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Mutagenesis and Inducible Responses to Deoxyribonucleic Acid Damage in *Escherichia coli*

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INTRODUCTION

The past several years have seen an explosive growth in our understanding of the molecular strategies employed by cells in responding to challenges to their genetic material. For no other organism do we have as complete a picture of the nature and regulation of the responses to deoxyribonucleic acid (DNA) damage as we currently have for *Escherichia coli*. In this review, I will attempt to provide an integrated view of these responses and will emphasize particularly the mechanisms involved in the introduction and avoidance of mutations as a consequence of chemical damage to the DNA of the cell.

PROCESSING OF DAMAGED DNA

DNA damage presents a very serious challenge to a cell because of the possibilities that it may give rise to mutations or lead to cell death. The evidence available to date suggests that, at least for *E. coli*, DNA lesions fall into two classes on the basis of the processing necessary to cause mutations. The first and smaller class, exemplified by O^6-methylguanine, appears to give rise to mutations by simply mispairing during normal DNA replication (78, 82, 208, 209). In contrast, the second and much larger class, which is exemplified by lesions introduced by agents such as ultraviolet (UV) radiation, methyl methanesulfonate, 4-nitroquinoline-1-oxide, and aflatoxin B1, seems to require the participation of a special inducible system to cause mutations (277, 371). I will use the term "SOS processing" to refer to this inducible system that is required for the introduction of mutations as a consequence of DNA damage; the genetics, molecular biology, and possible biochemistry of SOS processing will be discussed in detail in the latter half of this review.

Cells have evolved a wide variety of conceptually different strategies to repair damaged DNA accurately (110, 122, 130, 132, 192, 307). The simplest are those which directly reverse the damage without the need for the synthesis of any new phosphodiester bonds. The known examples of such processes are the photoreactivation of UV-induced pyrimidine dimers by photoreactivating enzyme (337) and the removal of the methyl group from O^6-methylguanine by O^6-alkylguanine-DNA transferase (192, 193, 259). Another type of accurate repair process is excision repair, which takes advantage of the fact that the information in DNA is present in two copies as a consequence of the complementary double-stranded nature of DNA. An incision is introduced into the damaged strand at or near the site of the lesion, a DNA fragment which includes the damage is excised, and the missing DNA is then resynthesized by using the opposite strand as a template (122, 132); the initiating incision event may result directly from the action of a damage-specific endonuclease such as the UvrABC endonuclease (295) or it may result from the sequential action of a damage-specific glycosylase and an apurinic/apyrimidinic (AP) endonuclease (122, 192). A third class of repair process found in *E. coli* termed "postreplication repair" or "daughter-strand gap repair" involves a recombination interchange of strands and appears to be a tolerance mechanism for dealing with gaps generated in daughter strands when advancing replication forks encounter DNA lesions (131, 132, 289). In principle, daughter-strand gap repair can occur in an error-free way, and at least some of the possible pathways for daughter-strand gap repair do not seem to result in the introduction of mutations.

The mutational consequences of DNA damage depend strongly on the nature and timing of the processing that the damaged DNA template undergoes. In recent years it has become clear that *E. coli* has at least three different regulatory networks of genes whose expression is induced in response to treatments with DNA-damaging agents. The products of these induced genes have various roles in accurate DNA repair, in SOS processing, and in other functions not necessarily related to DNA repair and mutagenesis. Since the fate of a particular DNA lesion is dependent on which regulatory networks have been induced and to what extent, it is easiest to open this discussion of mutagenesis by a consideration of the regulatory networks that are induced by DNA damage.

SOS REGULATORY NETWORK

Exposure of *E. coli* to agents or conditions that either damage DNA or interfere with DNA replication results in the increased expression of genes that are members of the SOS regulatory network. This was the first regulatory network induced by DNA damage to be recognized, and to date it is the only one whose regulation is understood in some detail at the molecular level. It is also a network whose expression profoundly affects the mutagenic consequences of a variety of different DNA-damaging treatments.

The existence of the SOS system was first hypothesized to account for the fact that a variety of diverse physiological phenomena that appear after DNA damage seemed to be under the coordinate control of two genes termed *recA* and *lexA*. This hypothesis was first clearly articulated by Defais et al. (70) and was later discussed in more detail by Radman (276, 277). The physiology of the SOS responses and the earlier work on the genetics of *recA* and *lexA* were reviewed in this journal by Evelyn Witkin (371) in an important and comprehensive article which served to focus much of the thinking in this area.

The elucidation of the regulatory mechanisms of the SOS network at the molecular level was aided greatly by the cloning of the two regulatory genes, *recA* and *lexA*, and by biochemical characterization of their gene products. The identification of genes that are members of the regulatory network was made possible by the use of genetic and in vitro operon fusion technology, which allowed studies of regulation to be uncoupled from studies of function. Aspects of these more recent advances in our understanding of the regulation of the SOS system have been reviewed comprehensively by Little and Mount (202) as well as by Kenyon (169), Gottesman (117), Echols (87), and Witkin (372).

Present Model for SOS Regulation

The basic regulatory mechanism of the SOS system is now understood at a molecular level and is diagrammed schematically in Fig. 1. In an uninduced cell, the product of the *lexA* gene acts as a repressor for a considerable number of unlinked genes, including the *recA* and *lexA* genes, by binding to similar operator sequences in front of each gene. Some loci, such as *recA*, have a single operator site which binds *lexA*, whereas others, such as *lexA* and *umuDC*, have two such operator sites. Many of these SOS genes, including the *recA* and *lexA* genes, are expressed at significant levels even in the repressed state. The amount of RecA protein present is evidently sufficient for the role(s) of RecA in homologous recombination (160, 291).

When a cell's DNA is damaged or its DNA replication is inhibited, an inducing signal is generated. The inducing signal reversibly activates a specific protease activity of RecA which allows it to cleave the LexA protein as well as a few other proteins such as the lambda repressor. The activated RecA protein cleaves the LexA protein at an —alanine—glycine— peptide bond near the middle of the protein to yield two proteolytic fragments. As the RecA protease cleaves LexA molecules, the pools of LexA begin to decrease so that various SOS genes, including the *recA* gene, begin to be expressed at an increased level, and SOS responses mediated by the products of these genes begin to be observed. Genes with operators that bind LexA relatively weakly are the first to turn on fully. If the inducing treatment was sufficiently strong, more molecules of RecA are activated and more molecules of LexA are cleaved. As the pools of LexA decline to very low levels, even genes whose operators bind LexA tightly are expressed at their maximal level.

As the cell begins to recover from the inducing treatment, e.g., by DNA repair, the inducing signal is eliminated, and the RecA molecules return to their proteolytically inactive state. In the absence of the RecA protease, the continued synthesis of LexA molecules now leads to an increase in the LexA pools. This in turn leads to repression of the SOS genes and a return to the uninduced state.

The basic elements of this model for SOS regulation seem to be relatively well established. Nevertheless, it is highly likely that additional genes will be shown to be members of the SOS network and that additional subtleties in the regulation of SOS genes will be discovered. To some extent, the approaches used in the analysis of the regulation of the SOS network can serve as paradigms for investigations of other complex regulatory systems.

SOS Responses

A considerable number of the diverse physiological responses of *E. coli* that appear after DNA damage or interfer-

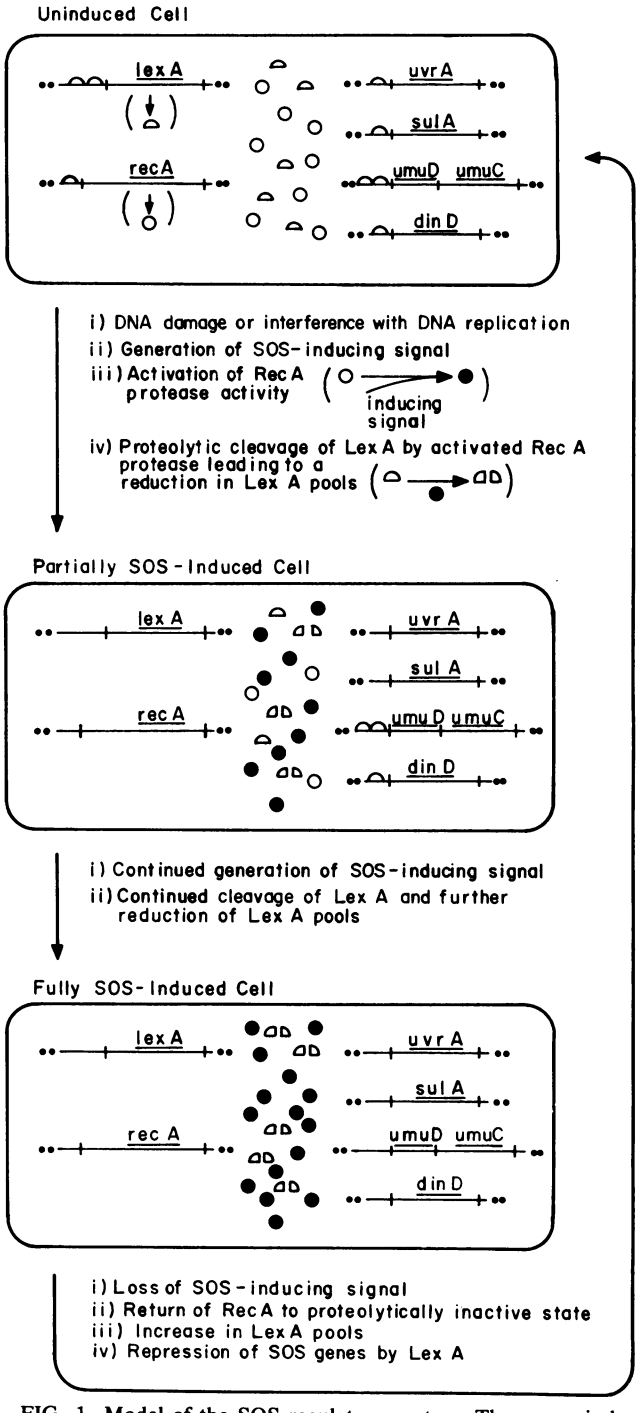

FIG. 1. Model of the SOS regulatory system. The open circles represent proteolytically inactive RecA molecules and closed circles represent proteolytically active RecA molecules. The semicircles represent LexA molecules.

ence with DNA replication have been classified as SOS responses, and many of these are summarized in Table 1. They share the common characteristic of being regulated by the RecA gene product and directly or indirectly by the LexA gene product. Of particular interest to this review is the fact that both UV mutagenesis of bacteriophage such as lambda (Wiegle mutagenesis) and UV mutagenesis of the chromosome are inducible functions. As will be discussed more fully below, these responses require the products of the *umuD* and *umuC* genes, and the *umuDC* locus is one of the operons controlled by the SOS regulatory network (3, 92). In addition, several loci on naturally occurring plasmids have been shown to be controlled by the SOS regulatory system.

The relationship between the physiology of the SOS responses and the induction of specific genes is gradually being established. For some responses, such as filamentation (146) and Weigle mutagenesis of UV-irradiated bacteriophage (3, 166), at least some of the genes whose products are required for the response have been identified. In other cases, genes whose products have known functions, for example *himA* (229, 230) and *uvrD* (137, 258, 263), are known to be induced by SOS inducing treatments, yet no induced physiological responses have been described that require the functions of those genes. Even less is known about other SOS-regulated loci such as *dinA* or *dinB* in which neither a biochemical function nor a phenotype has been associated with the inducible locus (171). For a number of SOS responses, such as alleviation of restriction, the induced genes required for the responses have not yet been identified.

The relationship between a physiological response and a gene function can be complex. Certain subsets of SOS responses such as Weigle mutagenesis of UV-irradiated bacteriophage and UV mutagenesis of the bacterial chromosome seem to have similar genetic requirements and thus may represent different physiological manifestations of the same induced pathway (166, 333, 371). Other sets of apparently different SOS responses could turn out to be related at a biochemical level. Studies of the genetic dependence of at

TABLE 1. SOS responses and genes in *E. coli*

Induced physiological response or gene function	Induced genes	Reference(s)
E. coli		
Prophage induction	Prophage genes	32, 136
Weigle reactivation of bacteriophage	*umuDC*, *uvrA*, *B*, and *C*	3, 70, 92, 166, 172, 233, 333, 363
Weigle mutagenesis of bacteriophage	*umuDC*, *recA*	3, 92, 70, 166, 333, 363
UV mutagenesis of bacterial chromosome	*umuDC*, *recA*	3, 92, 126, 166, 333, 369
Filamentation (inhibition of cell)	*sulA* (*sfiA*)	56, 146
$uvrA^+$ B^+ C^+-dependent repair	*uvrA*, *-B*, and *-C*	102, 172, 297, C. A. van Sluis, personal communication
Long patch repair	*uvrA*, *-B*, and *-C*, ?	57, 58
RecF-dependent recombination	*recN*	1, 207, 210
Induction of stable DNA replication	?	174, 186
Alleviation of restriction	?	67, 76, 343
Excision of element el4	?	40, 121
Cessation of respiration	?	340
Hyper-recombination in *uvrD* strains	?	206
Inhibition of DNA degradation by exonuclease V	*recA*	270
Induced radioresistance	*recA*	269
Repair of double-strand breaks	*recA*	35, 177
Induction of RecA protein (roles of homologous recombination; specific protease involved in SOS regulation)	*recA*	26, 125, 126, 203, 219
Induction of LexA protein (repressor; roles in SOS regulation)	*lexA*	25, 26, 198, 200
Induction of HimA protein (part of integration host factor; role in site specific recombination)	*himA*	229
Induction of UvrD protein (helicase II; roles in excision repair and methyl-directed mismatch repair)	*uvrD*	263, 328
Induction of single-strand DNA-binding protein	*ssb*	22
Induction of *ruv* locus (unknown role in UV resistance)	*ruv*	327
Induction of *dinA* locus (function unknown)	*dinA*	170, 171
Induction of *dinB* locus (function unknown)	*dinB*	170, 171
Induction of *dinD* locus (function unknown)	*dinD*	170, 171
Induction of *dinF* locus (in same operon as *lexA*)	*dinF*	170, 171, 178
Naturally occurring plasmids		
Increased susceptibility to UV and chemical mutagenesis, increased resistance to UV killing, increased Weigle reactivation	*mucAB* (pKM101)	93, 265, 354
Increased susceptibility to UV mutagenesis and increased resistance to UV killing	*imp* (TP110)	80
Colicin production	Colicin E1 (ColE1)	84, 85, 342
Colicin production	Colicin E2 (ColE2)	141
Colicin production	Colicin Ib (TP110)	P. Strike, personal communication
Cloacin production	Cloacin DF13 (CloDF13)	349

least one SOS response, Weigle reactivation of UV-irradiated bacteriophage, have indicated that the physiological response consists of at least two components, a *umuDC*$^+$-dependent component (166, 334) and a *uvrA*$^+$-dependent component (172); it is possible that other SOS responses may turn out to exhibit similar complexity. Particularly difficult types of responses to study are those in which the RecA gene product plays more than one role. For example, as will be discussed below, the RecA protein is involved in UV and chemical mutagenesis in at least two ways; it regulates the *umuDC* locus and also plays a second role which may be mechanistic. Similarly, the RecA protein plays two roles in stable DNA replication; one in the induction of stable DNA replication (174) and a second in the mechanism of stable replication (186).

Development of the Model for SOS Regulation

Genetic studies of *recA* and *lexA*. The initial evidence for the coordinate expression of the SOS responses grew out of genetic studies of the two key regulatory genes, *recA* and *lexA*. The first types of mutations identified were *recA* and *lexA*(Ind$^-$), each of which had the effect of preventing the induction of the set of responses now termed SOS responses (143, 252, 276, 277, 371). *recA* mutations were found to be recessive to *recA*$^+$, suggesting that the RecA product functioned as a positively acting control element in SOS regulation. In addition to its role in SOS regulation, the RecA product plays an essential role in homologous recombination in *E. coli* (51), and biochemical activities of RecA have been identified which are probably involved in homologous recombination (221, 325, 365); *recA* mutants are completely deficient in homologous recombination (51). In contrast, *lexA*(Ind$^-$) mutants were found to be dominant to *lexA*$^+$ (252), suggesting that the *lexA* product acted negatively in SOS regulation (44, 252). Unlike *recA* mutations, *lexA*(Ind$^-$) mutants were recombination proficient, a property which indicated that a deficiency in SOS induction was not necessarily associated with a deficiency in homologous recombination.

The phenotypes of mutations located at the *recA* and *lexA* loci were often found to be complex and difficult to interpret, a fact reflected in the many names that have been used in the literature to describe alleles of these genes. The properties of most of these mutations can now be explained relatively easily in terms of the model described above. Since it will be necessary to refer to a number of these *recA* and *lexA* alleles later while discussing mutagenesis, Table 2 summarizes the properties of these mutations.

There are now three classes of *lexA* alleles which have been identified, *lexA*(Ind$^-$) (formerly called *lexA*$^-$), *lexA*(Ts) (formerly called *ts1*), and *lexA*(Def) (formerly called *spr*). Unlike mutations in the first class, which are dominant and lead to a defect in the induction of the SOS responses, mutations in the other two classes are recessive and lead to constitutive expression of at least some of the SOS responses. (i) *lexA*(Ind$^-$) mutations (143, 252) alter the LexA protein in such a way that it is more resistant to proteolytic cleavage (198). In fact, the widely used *lexA3*(Ind$^-$) mutation changes the sequence at the —alanine—glycine— protease cleavage site (141) to —alanine—aspartate— (215). (ii) *lexA*(Ts) mutants were originally isolated as UV-resistant temperature-sensitive revertants of a *lexA*(Ind$^-$) strain (253). A *lexA*(Ts) mutation appears to result in the synthesis of a LexA protein which has a temperature-sensitive defect in its ability to function as a repressor for the SOS genes. The thermosensi-

TABLE 2. *lexA* and *recA* mutations affecting SOS induction

Alleles[a]	Phenotypes	Biochemical change	Reference(s)
lexA alleles			
lexA(Ind$^-$) (*lexA*$^-$)	Dominant, defective in SOS induction, UV sensitive	Protease-resistant LexA protein	143, 202, 215, 252
lexA(Ts) (*ts1*)	Recessive, expresses SOS responses if shifted to 42°C	Thermosensitive LexA protein, does not function well as repressor at 42°C	253
lexA(Def) (*spr*)	Recessive, constitutively expresses LexA-repressed genes	Defective LexA protein	178, 249, 262
recA alleles			
recA(Def) (*recA*$^-$)	Defective in recombination, SOS induction, and lambda induction; very UV sensitive	Defective in all activities of RecA protein	51, 99, 202, 277, 281, 371
recA441(*tif-1*)	At 42°C, expresses SOS functions constitutively and induces lambda prophage	Protease activity of RecA more easily activated	44, 219, 250, 268
recA730	Similar to *recA441* except expresses SOS function constitutively at 30°C	Protease activity of RecA much more easily activated	373
recA430(*lexB30*)	Recombination proficient	Defect in protease activity, does not cleave lambda repression, reduced ability to cleave LexA	93, 116, 242, 281, 284
recAo	*cis*-dominant constitutive *recA* expression	Operator constitutive mutation	52, 114, 350
recA453(*zab-53*)	Deficient in recombination and SOS induction	Promoter-down mutation	42, 53

[a] The nomenclature follows that of Little and Mount (202). I have used the term *recA*(Def) to describe *recA*$^-$ mutations. Names in parentheses are those used originally.

tivity of such mutants is due to lethal filamentation caused by constitutive expression of the LexA-repressed *sulA* (formerly called *sfiA*) gene (Table 1) and is not observed if the strains carry a *sulA* mutation (112). (iii) *lexA*(Def) mutants are unable to synthesize LexA molecules which can function as a repressor of the SOS genes. Missense mutations (249), amber mutations (262), and Tn*5*-generated insertion mutations (178) of the *lexA*(Def) type have now been isolated. In retrospect, it is clear that early genetic studies of the *lexA* locus did not yield mutants of the *lexA*(Def) type since the constitutive expression of the *sulA* gene would have been lethal. It was an awareness of this issue which led Mount (249) to carry out his successful search for a mutant of the *lexA*(Def) type in a *sulA* background (112), and all subsequent work with mutants of this type has been carried out in a *sulA* background.

Five classes of *recA* mutations are described in Table 2, and a few of them require additional comment. (i) The most common type, which I will refer to as *recA*(Def), causes a defect in both SOS induction and homologous recombination (51, 276, 371). Missense, amber, insertion, and deletion mutations of this type have been isolated, so it is clear that these are the phenotypes caused by *recA* null mutations (42, 248). Such mutants either produce no RecA protein or synthesize a defective RecA protein which can neither catalyze the proteolytic cleavage of LexA nor participate in reactions thought to be required for homologous recombination. (ii) Another class of *recA* mutation, represented by the intensively studied *recA441* allele (formerly called *tif-1*) (44, 45, 219, 250), leads to constitutive expression of SOS genes at 42°C; this effect is potentiated by the presence of adenine and antagonized by the presence of cytidine and guanosine by mechanisms which are still not understood. Biochemical studies of the RecA441 protein, which have indicated that its protease function is more easily activated than that of the wild-type RecA protein, have led to the suggestion that the RecA441 protein is able to activate its protease function by recognizing low amounts of SOS-inducing signal in uninduced cells which are not sufficient to activate the normal RecA protein (220, 268). A related allele, *recA730*, has recently been described which seems to resemble the *recA441* allele in many ways except that it causes constitutive expression of SOS responses even at 30°C (373). (iii) Another class of *recA* mutation, represented by the *recA430* allele (formerly called *lexB30*), does not cause a major defect in homologous recombination. It does, however, cause a defect in the induction of at least some of the SOS responses while not preventing the induction of at least some SOS genes (93, 116, 242). Biochemical studies of the RecA430 protein have indicated that it is very defective in cleaving lambda repressor and partially defective in cleaving LexA (281, 284), yet it can bind to single-stranded DNA. Thus, it seems likely that for a given SOS-inducing dose a *recA430* mutant is not able to deplete its LexA pools to the same extent as a $recA^+$ cell with the consequence that a less complete state of SOS induction is attained.

Deduction of the essential elements of SOS regulation. The specific functions of the *recA* and *lexA* gene products in the regulation of the SOS system were initially deduced from studies of two particular SOS responses: (i) lambda induction and (ii) the induction of an approximately 40,000-dalton protein termed protein X, which later turned out to be the RecA protein. These responses were particularly amenable to study since the consequences of induction could be measured directly rather than having to be inferred from more complicated physiological responses.

The insight that proteolytic cleavage of a repressor could be involved in SOS regulation first came from studies of lambda induction by Roberts and Roberts (283). They showed that treatment of a lambda lysogen with UV or mitomycin C resulted in the proteolytic cleavage of the lambda repressor and that this breakdown of the repressor correlated with the expression of phage genes. Since both lambda induction and the induced cleavage of the lambda repressor could be blocked by *recA*(Def) mutations, it was suggested that the RecA protein played a role in this process either by regulating a protease or by being a protease itself.

About the same time, Gudas and Pardee (124, 126, 127) showed that the induction of the synthesis of protein X by nalidixic acid could be blocked by *lexA*(Ind^-) mutations as it could be by *recA*(Def) mutations (148). Furthermore, the synthesis of protein X could be induced by simply shifting a *lexA*(Ts) mutant, a *recA441* mutant, or a *lexA*(Ts) *recA*(Def) double mutant to an elevated temperature. On the basis of these observations, they proposed that LexA repressed the gene coding for protein X and possibly other SOS genes and that the RecA protein was involved in the inactivation of LexA. Because of the observations of Roberts and Roberts that lambda repressor was proteolytically cleaved at the time of SOS induction, the possibility was raised that LexA was also inactivated by proteolytic cleavage. The following year, McEntee and others (94, 125, 201, 219) demonstrated that protein X was actually the *recA* gene product and thus that RecA played a role in its own induction.

The first *lexA*(Def) mutant was isolated by Mount (249) by screening for a derivative of a *lexA*(Ind^-) *recA441* strain that could not be lysogenized by lambda. Strains carrying this *lexA*(Def) mutation also constitutively expressed other SOS responses including the high-level synthesis of the RecA protein (125). Of particular importance was the observation that *lexA*(Def) *recA*(Def) double mutants constitutively synthesized high levels of the RecA protein, a genetic observation that strongly suggested that LexA functioned as a repressor of the *recA* gene and that the RecA protein was involved in the inactivation of the LexA protein at the time of SOS induction.

The protease that inactivated the lambda repressor was isolated by Roberts et al. (285, 286) from a strain carrying a *lexA*(Def) mutation, and the protease was then shown to be the product of the *recA* gene.

Since the RecA protein itself was capable of expressing a proteolytic activity and genetic studies suggested that RecA was involved in the inactivation of LexA, it seemed likely that the LexA protein was also cleaved by the RecA protease at the time of SOS induction. This key element of the control circuit was established when the *lexA* gene product was identified as a 24,000-dalton protein by Little and Harper (200) and by Brent and Ptashne (25) and was shown to be proteolytically cleaved in vitro by the RecA protease in a fashion similar to the lambda repressor (141, 198). The purified LexA protein was then biochemically shown to function as the direct repressor of both the *recA* gene and the *lexA* gene (26, 203).

J. W. Little (personal communication) has recently observed that, in the absence of RecA protein, both highly purified LexA protein and lambda repressor are capable of a specific type of autodigestion that leads to the cleavage of the same bond that is cleaved in the presence of RecA. These results raise the possibility that the RecA protein plays an indirect stimulatory role, perhaps as an allosteric effector in the RecA-dependent reaction, rather than acting directly as a protease. For the purpose of this review I will

refer to RecA as being a protease; however, the logic of the control circuit is the same whether RecA is acting directly as a protease or is stimulating a specific autodigestion of LexA, lambda repressor, and related proteins.

Identification of genes in the SOS network. The experiments described above demonstrated the manner in which the RecA and LexA gene products regulate the *recA* and *lexA* genes and control the induction of lambda. However, they did not indicate how SOS-inducing treatments lead to the expression of the other SOS responses. The most obvious possibility was that SOS induction led to the increased expression of a set of genes controlled by RecA and LexA; a number of studies showing that inhibitors of transcription or translation interfered with the induction of various SOS responses tended to support this view. However, the discovery of the protease activity of the RecA protein raised at least one plausible alternative, that the SOS responses were due to the proteolytic modification of preexisting proteins by RecA; the requirement for protein synthesis at the time of SOS induction would then just be for RecA synthesis rather than for the synthesis of other genes. Analysis of the regulation of these other SOS functions was complicated by the physiological complexity of many of the responses.

In an effort to dissociate the physiological complexity of the SOS responses from the issue of their regulation, Kenyon and Walker (171) took advantage of the Mu dl(Ap *lac*) bacteriophage, a powerful tool constructed by Casadaban and Cohen (41), which makes it possible to construct operon fusions in vivo in a single step (179). The bacteriophage carries the structural genes for the *lac* operon near one of its ends, but no promoter for these genes. Mu dl(Ap *lac*) integrates into the *E. coli* chromosome essentially at random; however, β-galactosidase can only be expressed if the bacteriophage inserts into a chromosomal transcriptional unit in the correct orientation.

The first question addressed was whether there was a set of genes whose expression was increased in response to SOS-inducing treatments. A set of random Mu dl(Ap *lac*)-generated fusions in the *E. coli* chromosome was screened, searching for fusions which expressed β-galactosidase at higher levels in the presence of the DNA-damaging agent mitomycin C than in its absence. By this procedure, a set of *din* (damage-inducible) loci were identified whose expression was increased by a variety of SOS-inducing treatments (Fig. 2). The induction of these loci was blocked by *recA*-(Def) and *lexA*(Ind$^-$) mutations, indicating that there were in fact a set of inducible loci in the *E. coli* chromosome that were controlled by the *recA*$^+$ *lexA*$^+$ regulatory circuit. The screen carried out is a specific example of a general approach made possible by the Mu dl(Ap *lac*) phage and other techniques for conveniently constructing fusions. One is able to search for genes which are members of a common regulatory network without having to know the functions of the genes in advance.

Further genetic tests of the regulation of these fusions were consistent with the *din* genes being repressed by LexA and with the RecA product being required for the inactivation of LexA. Particularly important were the observations that the *din-lac* fusion strains expressed β-galactosidase constitutively at a high level whether they carried a *lexA*-(Def) mutation or both *lexA*(Def) and *recA*(Def) mutations (170, 172, 362). The result that the *din* loci were expressed at high levels in the absence of a functional LexA protein

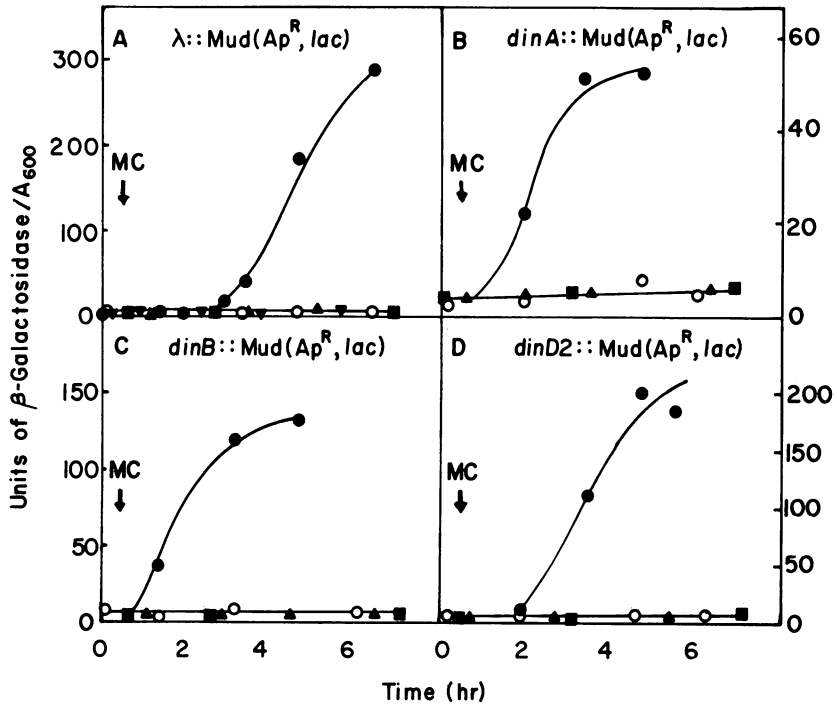

FIG. 2. Kinetics of induction of β-galactosidase in *din-lac* fusion strains (17). The *din-lac* fusions were generated by the insertion of the Mu dl(Ap *lac*) bacteriophage into the *E. coli* chromosome. The λ::Mu dl(Ap *lac*) derivative was generated by an insertion of Mu dl(Ap *lac*) into a lambda prophage in the *E. coli* chromosome. Symbols: ○, untreated fusion strains; ●, fusion strains plus mitomycin C; ▲, *lexA*(Ind$^-$) derivatives of the fusion strains plus mitomycin C; ■, *recA*(Def) derivatives of the fusion strains plus mitomycin C; ▼, a pKM280-containing derivative of the λ::Mu dl(Ap *lac*) strain plus mitomycin C.

suggested that LexA was acting as a repressor of these loci. Moreover, the results suggested that the role of RecA protein in *din* gene regulation was to inactivate LexA, since strains which lacked a functional LexA no longer required a functional RecA protein to express the *din* genes at high levels.

One of the *din* mutants identified in this initial screen was as sensitive to UV killing as *uvr* mutants, which are defective in excision repair, and the Mu dl(Ap *lac*) insertion in this strain mapped at the position of *uvrA* (171). Further characterization indicated that the *lac* fusion was indeed to the *uvrA* gene, and thus that at least one of the SOS-regulated loci identified by this procedure actually coded for a gene product (172). The observation that the *uvrA* gene was inducible was consistent with a series of in vivo observations indicating that uvr^+-dependent repair processes can be induced in *E. coli* (57, 58, 319, 320).

Subsequently, the Mu dl(Ap *lac*) bacteriophage played an important role in studies by a variety of investigators in helping to identify genes which are members of the SOS regulatory network. Rather than being used first to search for genes on the basis of their regulatory characteristics as in the experiment described above, the bacteriophage has instead been used to generate insertion mutations in genes suspected of being members of the SOS system. Then, after an insertion of the bacteriophage in the gene of interest has been obtained, the regulation of the gene has been investigated by examining expression of β-galactosidase. By this strategy, a number of genes have been shown to be controlled by the SOS system including: *uvrA* (172), *uvrB* (102, 172), *sulA* (146), *umuDC* (3), *himA* (229), *uvrD* (328; H. M. Arthur and P. T. Emmerson, personal communication), *ruv* (327), *recA* (42), and *recN* (207). Genetic analyses of the regulation of these were again consistent with LexA serving as the repressor of each of these genes. In addition, in vitro fusion techniques have been used to study the regulation of the *lexA* (25), *uvrC* (C. A. van Sluis, personal communication), *ssb* (22), and *umuDC* (92, 326) genes as well as the plasmid-encoded *mucAB* genes (93).

Besides the use of fusions, examinations of the effects of LexA on the level of proteins synthesized in maxicells have also been used to demonstrate LexA control of particular genes (92, 93, 263). Immunological techniques have been used to analyze the regulation of the *recA* (160, 291) and *ssb* genes (292). In the case of *ssb*, fusion techniques and immunological techniques have led to conflicting results regarding the inducibility of *ssb*. The regulation of transcription of SOS genes has also been analyzed by following in vitro transcription (222; D. W. Mount, personal communication).

As of the writing of this review, 17 chromosomal genes (Fig. 3) have been shown to be members of the SOS regulatory network, and it seems likely that additional ones will be identified. To put this in some perspective, this means that on the order of 0.5% of the genes in *E. coli* are part of this complex regulatory system.

Biochemical studies of SOS regulation. Genetic analyses of the regulation of the genes that are members of the SOS regulatory network have been consistent with LexA being the direct repressor of each. This genetic prediction has been confirmed by biochemical approaches for seven of these genes. Purified LexA protein has been shown to inhibit transcription in vitro or to bind to the operator sequences of the *recA* (26, 203), *lexA* (26, 203), *uvrA* (297), *uvrB* (296), *uvrD* (A. M. Easton and S. R. Kushner, personal communication), *dinA* (170), and *dinB* (170) genes of *E. coli* as well as to the operator sequence of the plasmid-encoded *cle*1 gene (85). Comparison of the sequences of the operators identified in these footprinting experiments revealed that there was considerable homology among them (202). Several *recA* mutants which were classified as operator-constitutive mutants were shown to have a base pair change within the region containing this homologous sequence (52, 346). Since then, the promoter/operator regions of a number of SOS genes have been sequenced, and homologous sequences have been found in all of them (Table 3). The strength with which LexA binds to these different operators varies considerably. For example, the K_d values for binding to the *recA* operator sequence in 2 nM whereas the K_d values for binding to the *uvrB*, *lexA* and *cle*1 operator sequences are approximately 20, 20, and 0.04 nM, respectively (26, 86). Physical aspects of the LexA operator interaction have been investigated, and it appears that the LexA protein binds a region 18 base pairs in length, interacting at least with bases in the major groove (24).

More detailed biochemical studies of the LexA protein have suggested that it has strong functional similarities to the lambda repressor. For example, both molecules are cleaved at an —alanine—glycine— bond in the middle of the polypeptide (141, 215). Furthermore, an evolutionary relationship between these two molecules is suggested by homologies in their protein and nucleic acid sequences (141, 142, 215, 301). The DNA binding activity of the LexA protein appears to reside in its amino terminus, since truncated LexA proteins produced either by recombinant techniques (24, 142) or by insertion mutations (178) appear capable of at least partially repressing SOS genes in vivo.

SOS-Inducing Signal

Central role of RecA activation. In vitro studies have indicated that RecA is the only protein that need be present for cleavage of the LexA protein to occur (141, 198, 203). Uninduced *E. coli* cells contain approximately 800 to 1,200 RecA molecules per cell (160, 291), and consistent with the in vitro result, a variety of in vivo observations have suggested that in SOS-induced cells at least some cleavage of

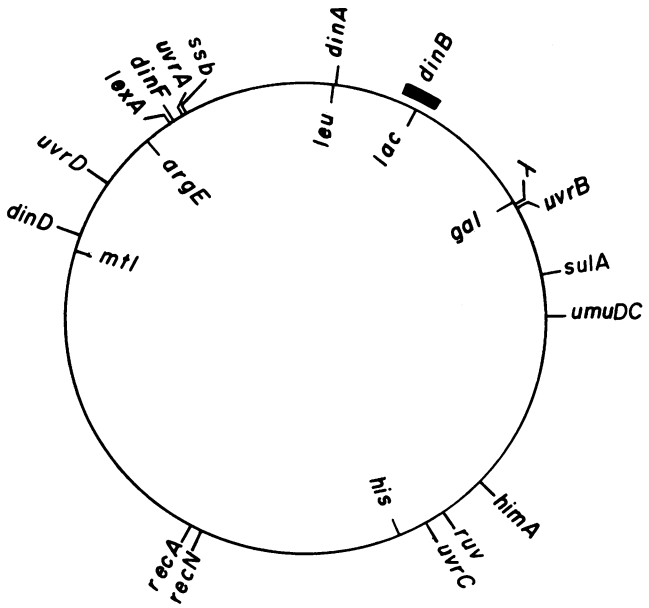

FIG. 3. Chromosomal location of genes which are members of the SOS regulatory network.

TABLE 3. LexA binding sites of SOS genes

Gene	Sequence	Reference(s)
	A[a] G[a] G[a]	
	↑ ↑ ↑	
recA	TA*CTG*TATGAGCATA*CAG*TA	26, 203
uvrA	TA*CTG*TATATTCATT*CAG*GT	297
uvrB	AA*CTG*TTTTTTTATC*CAG*TA	296
sulA[b]	TA*CTG*TACATCCATA*CAG*TA	56
uvrD	AT*CTG*TATATATACC*CAG*CT	A. M. Easton and S. Kushner (personal communication)
mucAB[b]	TA*CTG*TATAAATAAA*CAG*TT	B. B. Mitchell, K. L. Perry, and G. C. Walker, unpublished data
clo13[b]	TA*CTG*TGTATATATA*CAG*TA	349
himA[b]	TC*CTG*CA AGATACC*CAG*GC	H. I. Miller (personal communication)
lexA-1	TG*CTG*TATATACTCA*CAC*GA	26, 142, 203, 227
lexA-2	AA*CTG*TATATACACC*CAG*GG	
*cle*1-1	TG*CTG*TATATAAAAC*CAG*TG	84, 86, 349
*cle*1-2	CA*GTGG*TTATATGTA*CAG*TA	
umuDC-1[b]	AT*CTG*CTGGCAAGAA*CAG*AC	B. B. Mitchell, S. J. Elledge, and G. C. Walker, unpublished data
umuDC-2[b]	TA*CTG*TATATAAAAA*CAG*TA	
Consensus	t a CTG T a t a t a - a - a CAG t a	

[a] The arrows indicate base pair changes in the *recA* operator which cause operator-constitutive mutations (52, 346).

[b] These sequences have homology to known LexA binding sites and are found upstream of genes known to be regulated by LexA. However, the sequences have not yet been shown to function as LexA binding sites in in vitro experiments.

LexA and lambda repressor can occur without RecA synthesis taking place (7, 197, 243, 318).

For the proteolytic activity of RecA to be expressed, the protein must be activated; simply overproducing RecA is not sufficient to cause the induction of the SOS responses (52, 274, 345). The activation is reversible (43, 197) and is thought to involve a conformational change of the RecA molecule (281). In vitro, the activation of RecA occurs when it forms a ternary complex with single-stranded DNA and a nucleoside triphosphate (61, 62, 281, 282). It is still unclear exactly what constitutes the inducing signal in vivo. On the basis of the in vitro results, however, it seems reasonable that single-stranded regions of DNA generated by SOS-inducing treatment form at least part of the in vivo signal for SOS induction.

Agents and treatments which cause SOS induction. A wide variety of chemical agents have been shown to induce the SOS responses (3, 273). These agents probably generate single-strand regions of DNA by introducing lesions which block or delay replication. Similarly, starving cells for thymine leads to blockage of replication. Roberts and Devoret (281) have suggested that RecA is activated when it binds to gaps caused by the replication fork encountering a lesion. The concentration of activated RecA protein and the overall rate of repressor cleavage would depend upon the linear density of lesions in the DNA which would in turn be a function of the dose of inducing treatment. Thus, activation of RecA would occur transiently at each lesion, and the intensity and duration of the activation would depend on the number of lesions introduced into the DNA. This model is consistent with the observations of Little that, after UV irradiation, there is a 1-min lag before LexA cleavage begins (197).

Indirect induction. This view is also supported by a variety of indirect induction experiments in which induction of the SOS system occurs upon the introduction of a UV-damaged DNA molecule such as F, F', P1, M13, λ, λ derivatives carrying part of the F replication system, or Hfr DNA (19, 20, 66, 73, 113, 288). An analysis of the efficiency of such damaged DNAs in inducing the expression of a *sulA-lac* fusion suggests that replication of the damaged template is not required to cause the generation of an SOS-inducing signal (66). Induction is more efficient, however, if such replication can occur. Furthermore, the fact that UV-irradiated lambda derivatives carrying a part of the F replication region are more efficient inducers of the SOS responses than UV-irradiated lambda itself (66, 244, 281) suggests that some replication systems may be more efficient at generating an inducing signal than others.

Role of DNA replication. The observations that SOS functions are induced by temperature shifts in *dnaB*(Ts) (371), *dnaE*(Ts) (312), and *dnaE*(Ts) *polA*(Ts) strains (13) are consistent with the notion that a stalled replication fork generates an inducing signal. It seems, however, that additional subtleties are likely to be involved. For example, blockage of DNA replication by shifting an initiation-defective *dnaA*(Ts) mutant to the restrictive temperature does not induce the SOS responses, yet such cells can be induced by mitomycin C and UV at the restrictive temperature (43, 199, 312). Similarly, shifting a *dnaC*(Ts) mutant to the nonpermissive temperature does not induce the SOS responses; however, such cells cannot be induced by mitomycin C at the higher temperature (43). It is possible that lesions generated in the DNA can serve as substitute origins for abortive *dnaC*$^+$-dependent initiation in the absence of *dnaA*. In addition, shifting a *dnaG*(Ts) mutant to the restrictive temperature does not induce the SOS network, although such cells express at least some SOS genes at a high basal level at the permissive temperature (43). Addition of nalidixic acid, a DNA gyrase inhibitor, blocks DNA replication, but SOS induction does not occur unless the cell is *recB*$^+$ *recC*$^+$, suggesting that for this agent the generation of an inducing signal requires the action of ExoV. Finally, SOS induction can occur as the result of aberrant forms of DNA replication (40, 281).

Strains containing a *recA441* mutation express SOS functions at 42°C in the absence of any DNA-damaging treatment. The RecA441 protein can be activated in vitro by a broader spectrum of oligonucleotides and polynucleotides and binds ATP more tightly than can the wild-type RecA protein (220, 268). It has been suggested that the RecA441 protein becomes activated by binding to regions of single-stranded DNA such as those at the replicating fork that are found in uninduced cells (282). The observation that the initiation-defective *dnaB252* allele suppresses RecA441-mediated induction raises the possibility that the RecA protein might be directly involved in the replication complex in processes leading to its activation (65).

Involvement of small molecules. Studies with permeabilized cells have shown that ϕ80 can be induced upon the

addition of specific small oligonucleotides (149, 150); however, these small oligonucleotides do not activate the RecA protease sufficiently to cleave lambda repressor in vitro (61, 62, 196, 268). Since the φ80 repressor is inferred to be more sensitive to proteolysis than the lambda repressor (74), this difference may simply be a result of differing sensitivities of the two systems. Alternatively, it may be the result of the participation of additional proteins in the activation process in the permeabilized cells. For example, the small oligonucleotides might activate a nuclease in the permeabilized cells which would then generate single-strand DNA. In any case, it seems somewhat unlikely that short oligonucleotides constitute a major inducing signal in vivo since *uvrB recB* mutants are fully and persistently induced by UV in spite of the fact that they undergo minimal UV-induced DNA degradation (14).

The role of nucleoside triphosphates in the generation of the in vivo inducing signal is unclear. RecA protein is most strongly activated in vitro for the cleavage of lambda repressor when the nucleoside triphosphate present is dATP (268). The observation that the SOS responses can be indirectly induced by the introduction of UV-irradiated DNAs even though normal chromosomal replication continues suggests that large shifts in the nucleoside triphosphate pools are unlikely to be the primary inducing event (66).

Role of single-strand DNA-binding protein. It is possible that other proteins besides RecA and LexA may play a role in the induction of the SOS system, either directly or indirectly. The most interesting possibility is the single-strand DNA-binding protein (SSB), the product of the *ssb* gene. Strains carrying either the *ssb-1* (224) or *ssb-113* (225) mutations have defects in a number of SOS responses including induction of lambda prophage (348), Weigle reactivation and Weigle mutagenesis (366), UV mutagenesis (189), increased synthesis of RecA protein (6, 226), and control of postirradiation DNA degradation (190).

The SSB protein isolated from an *ssb-1* strain binds more weakly to single-stranded DNA than does wild-type SSB, whereas SSB isolated from an *ssb-113* strain competes more effectively with RecA for binding sites on single-stranded DNA than does wild-type SSB (55, 366). At least in the case of *ssb-1*, it does not seem that the defects in SOS induction arise from some aberrant property of the mutant protein. Rather it seems that it is due to a defect in some function of SSB, since overproduction of the *ssb-1* gene restores the ability to induce the SOS system (47). This result suggests that SSB plays some positive role in the events leading to the cleavage of the LexA and lambda repressor proteins.

This conclusion is consistent with the in vitro results of Resnick and Sussman (279) who have shown that SSB increases the rate and extent of lambda repressor cleavage by RecA and that the maximal cleavage rate achievable with SSB exceeds the maximal rate achievable in its absence. In addition, higher concentrations of SSB protein from an *ssb-113* mutant interfere with lambda repressor cleavage in vitro, whereas wild-type SSB protein does not, probably because the mutant SSB protein competes more effectively than the wild type for binding to DNA sites necessary for RecA protein binding and activation (55). These results differ from those reported previously by Weinstock and McEntee (364), using somewhat different experimental conditions, who found that SSB at higher concentrations inhibits repressor cleavage. The more recent results suggest that SSB plays an active role in the process of LexA and lambda repressor cleavage rather than just a passive role of sequestering excess single-stranded DNA. This view is supported by the observation that overproduction of RecA protein in an *ssb-1* mutant does not suppress the deficiencies in SOS induction caused by the *ssb-1* mutation (190, 367).

The nature of the putative active role of SSB is not clear. Electron microscopy studies have shown that SSB accelerates the interaction of RecA protein with single-strand DNA (101), suggesting that the RecA protein may have increased affinity for the nucleotides adjacent to SSB. Analyses of the interaction of SSB, RecA, and single-strand DNA have led to the suggestion that, in vivo, the active machinery for SOS induction and for strand assimilation is a quaternary complex of SSB, RecA, single-strand DNA, and a nucleoside triphosphate (55). The major role of SSB in SOS induction seems to be to play a role in the activation of RecA, since *ssb* mutations do not prevent thermal induction of SOS function in *recA441* mutants (367) or the constitutive expression of SOS functions in *recA730* strains (190).

Fine Tuning in the Induction of the SOS System

Once a cell has been exposed to an SOS-inducing treatment, several factors influence whether a given SOS response will be expressed: (i) the amount of inducing signal that is generated and its persistence—this in turn influences the amount of RecA protein that is activated and the length of time that it stays activated, (ii) the rate at which the repressor of a particular SOS gene is proteolytically cleaved by activated RecA protein, (iii) the affinity of the repressor for the operator of a particular SOS gene, and (iv) the degree of expression of a given SOS gene that is required for its gene product to have a physiologically manifestable consequence.

Intermediate states of induction. It has become clear that although the SOS system can exist in two extreme states, fully repressed and fully induced, it can also exist in any of a number of other states which are intermediate between these two extremes. The state of SOS induction that results from a given SOS-inducing signal is influenced by the fact that the two key regulatory genes, *lexA* and *recA*, are themselves regulated. Since the *recA* gene is more strongly repressed by LexA than a number of other LexA-repressed genes (26, 203), the generation of only a low amount of inducing signal can lead to the high-level expression of some of the SOS functions without substantial amplification of the RecA protein. Larger amounts of inducing signal lead to the synthesis of increased amounts of RecA protein and thus to the possibility of even greater amounts of activated RecA if the inducing signal is not limiting.

Repression of the *lexA* gene by the LexA protein has three effects on SOS induction. First, it extends the range, in terms of inducing signal, over which the system can establish an intermediate state of induction and thus express only a subset of the SOS responses. Second, since the affinity of LexA for its own operator is weak relative to its affinity for the operator of the *recA* gene, the system is buffered against being substantially induced by very small amounts of inducing signal. Third, it speeds the return to the repressed state once the amount of inducing signal begins to decrease (24–26, 197, 203).

Since all of the chromosome- and plasmid-encoded SOS genes identified to date are repressed by LexA, their extent of induction is dependent on the degree to which the LexA pool has been decreased in response to a given SOS-inducing treatment. Those genes that bind LexA most weakly are fully turned on in response to even weak SOS-inducing treatments, whereas genes which bind LexA more strongly are only fully turned on in response to stronger SOS-

inducing treatments. As mentioned earlier, direct physical measurements of LexA binding have shown that LexA binds less strongly to the *lexA* and *uvrB* operators than to the *recA* operator (24, 26, 203), whereas genetic experiments have suggested that the *uvrA*, *dinA*, and *dinB* genes bind LexA less strongly than *umuDC* and that *umuDC* binds LexA less strongly than *dinD* (178). Thus, it seems that relatively weak SOS-inducing treatments would lead to increased expression of the *uvr*$^+$-dependent excision repair function. However, other SOS responses, such as the accumulation of large amounts of RecA and in induction of substantial amounts of *umuDC*$^+$-dependent mutagenesis/repair, would not occur unless the cell received a stronger SOS-inducing treatment. This feature of the regulatory system allows an *E. coli* cell to utilize certain SOS-regulated functions such as excision repair to recover from DNA damage without committing itself to a full-fledged SOS response.

Once the pools of LexA are low enough that it no longer represses a particular SOS gene, its expression could in principle be influenced by other factors in the induced cell such as the dependence of the strength of its promoter on superhelical density, the need for a transcriptional activator, or some form of attenuation. Furthermore, some of the more physiologically complex SOS responses may require the participation of gene products whose expression is not regulated by the SOS system, and the availability of these other gene products could influence the course of such SOS responses.

As a cell approaches the final state of SOS induction determined by the strength of the inducing signal, the various SOS genes are turned on fully in an ordered fashion. However, since the pools of LexA decrease very rapidly after an inducing treatment (197), the order in which the genes are turned on is probably of less physiological significance than the subset of SOS genes that is ultimately induced.

Factors influencing the extent of SOS induction. The ability of the SOS system to be induced to an intermediate state may account, at least in part, for a variety of observations in the literature in which particular SOS-inducing treatments only induced subsets of the SOS responses, phenomena referred to "split phenotypes" by Witkin in her 1976 review (371). Furthermore, for many years, the extent of SOS induction was gauged by monitoring the appearance of a physiological response rather than the direct expression of an SOS gene. This method of judging the extent of SOS induction may have exaggerated the effects taking place at a transcriptional level since the extent of a physiological response is not necessarily linearly related to the expression of the relevant SOS gene. The induction of subsets of the SOS responses by different inducing treatments or by the same inducing treatment in different mutants was confusing for a number of years since it was compatible with models for SOS regulation in which each gene in the network had its own individual repressor (371).

Situations in which SOS induction has been directly shown to proceed only to an intermediate state after a chemical treatment have been studied by monitoring LexA cleavage (24, 197) or lambda repressor cleavage (4, 7, 243).

Mutations which perturb the functions of the RecA or LexA gene products can also contribute to the production of intermediate states of SOS induction. For example, *recA430* mutants do not induce lambda prophage after SOS-inducing treatments, do degrade their DNA drastically after UV, and are nonmutable with UV (12, 116, 242). They are, however, able to induce φ80 lysogens (74), amplify RecA protein somewhat (74), and induce the expression of several SOS genes (93). Purified RecA430 protein does not cleave lambda repressor detectably in vitro (284), yet it is capable of cleaving LexA (281). Thus, the RecA430 protein appears to be impaired in its ability to cleave LexA proteolytically so that the same treatment that would lead to a full induction of the SOS system in a *recA*$^+$ strain only leads to an intermediate level of induction in a *recA430* strain. In another example, when a *lexA41* mutant is grown at 42°C only a subset of the SOS responses can be detected physiologically, suggesting that the LexA41 protein retains some residual repressor activity at the higher temperature and can still repress those genes to which it binds most tightly. Even at 30°C *lexA41*(Ts) mutants express a somewhat higher-than-normal level of RecA (42, 126), apparently since the LexA41 protein does not function as a repressor as well as the wild-type LexA protein.

The SOS system can also be induced to an intermediate level by the indirect induction experiments referred to in the preceding section. For example, the introduction of a UV-irradiated F factor into a cell leads to the induction of both Weigle reactivation and lambda prophage, yet the introduction of UV-irradiated Hfr DNA induces Weigle reactivation but not lambda prophage (19, 20, 73, 113). Similarly, infection with UV-irradiated lambda does not lead to lambda prophage induction, whereas infection with UV-irradiated bacteriophage P1 (288) or UV-irradiated lambda derivatives carrying F replication functions (281) does lead to lambda prophage induction. The experiments of D'Ari and Huisman (66) studying the effects of indirect induction on the expression of a *sulA-lac* fusion strongly suggest that the introduction of any piece of UV-irradiated DNA into a cell causes some induction of the SOS system. However, the extent to which the system is induced depends strongly on such factors as whether the irradiated DNA is capable of replication and the nature of the specific replication system.

Strategies for modulating individual genes within the SOS network. Although all of the cellular SOS genes identified to date are similarly regulated in that they are repressed by LexA, a variety of different strategies are used to fine tune the expression of individual genes within this large regulatory network.

For example, most of the SOS genes have a single binding site for LexA near their promoters, but the *lexA* (26, 203), *umuDC* (B. B. Mitchell, S. J. Elledge, and G. C. Walker, unpublished data) and colicin E1 (86) genes appear to have two adjacent LexA binding sites separated by 5, 4, and −1 base pairs, respectively. In the case of the *lexA* gene, the LexA protein has been shown to exhibit weak cooperative binding to these sites. The colicin E1 gene binds LexA cooperatively and very tightly. Such cooperativity has the effect of narrowing the range of LexA concentrations over which the genes are induced.

At least one of the SOS genes, *uvrB* (296), has two promoters, only one of which is regulated by LexA. The presence of the unregulated promoter leads to a relatively high basal level of expression of the gene product in the absence of an SOS-inducing treatment.

At least one of the genes in the SOS network is subject to a second type of regulation. The *himA* gene is not only repressed by LexA but is also autoregulated by its own gene product. The LexA binding site in front of the *himA* gene differs from the consensus sequence and is located immediately beside a binding site for integration host factor, one subunit of which is encoded by the *himA* gene (H. I. Miller, personal communication). The suggestion has been made

that LexA is only able to bind to this partially defective LexA binding site if an initiation host factor molecule is bound to the adjacent initiation host factor binding site. As our knowledge of the SOS system continues to expand, other genes in the network may be recognized as being subject to additional forms of regulation.

The repressors of a number of temperate bacteriophage such as lambda, 434, 21, P22, and φ80 (281) can be cleaved by the RecA protease. Prophage induction differs from the cellular SOS responses in being an irreversible process. Since the prophage are not repressed by LexA, the state of SOS induction at which they induce is determined both by the susceptibility of their repressor to RecA cleavage and by the affinity of the repressor for the prophage operator. The susceptibility of these repressors to RecA proteolysis seems to be φ80 > LexA > λ or 434 (74, 281). The susceptibility of a protein to cleavage by RecA may be determined not only by its amino acid sequence but also by its multimeric state since it is apparently the monomer of lambda repressor rather than the dimer which is cleaved by RecA (267). Mutants of lambda repressor that increase its susceptibility to cleavage by RecA allow the induction of lambda prophage under SOS-inducing conditions that are not normally strong enough to induce lambda (54, 63). Although all the chromosomal and plasmid-coded SOS genes recognized to date are repressed by the LexA protein, the possibility should be kept in mind that chromosomally or plasmid-encoded genes could be identified at some point in the future that are repressed by some RecA-cleavable protein besides LexA, i.e., a conceptual analog of one of the prophage repressors.

As mentioned above, other factors besides the removal of a repressor could influence the expression of particular genes in cells with damaged DNA. It is possible, for example, that the effects of *supX* (topoisomerase I) or *gyrB* (subunit of DNA gyrase) mutations on mutability and DNA repair (31, 261, 351) could be due to the dependence of the strength of particular promoters, such as the promoter of the *umuDC* locus, on superhelical density.

INDUCTION OF HEAT SHOCK GENES BY DNA-DAMAGING AGENTS

The response of *E. coli* to the SOS-inducing agents UV and nalidixic acid has recently been found to be more complex than simply the induction of the SOS system. In the course of characterizing the properties of *lexA*::Tn*5* mutants, Krueger and Walker (Proc. Natl. Acad. Sci. U.S.A., in press) observed that a 61,000- and a 73,000-dalton protein were induced by UV and nalidixic acid even though the cell contained no LexA protein. At first it seemed possible that the genes encoding these products might be part of the SOS system but repressed by a protein that was not LexA but could be cleaved by the RecA protease (i.e., a conceptual analog of the lambda repressor). However, further studies indicated that these proteins could be induced by these agents even in *recA*(Def) and *lexA*(Ind$^-$) strains and thus that they were not part of the SOS responses. Instead, analyses of these proteins by two-dimensional gel electrophoresis revealed that the 61,000- and 73,000-dalton proteins comigrated with the two heat shock proteins, GroEL and DnaK, respectively, and that the other heat shock proteins were also induced by UV and nalidixic acid. Furthermore, the induction of *groEL* and *dnaK* by UV and nalidixic acid was found to be controlled by the *htpR* gene product (Krueger and Walker, Proc. Natl. Acad. Sci. U.S.A., in press), a positively acting element required for expression of the heat shock genes (255, 256, 374). These results indicate that *E. coli* not only responds to UV and nalidixic acid by turning on the SOS system but also by inducing a second independent regulatory system, the heat shock response.

The physiological significance, if any, of the induction of the heat shock proteins by these agents is still unknown as is the nature of the inducing signal for this system. However, I will speculate that *htpR*-controlled gene products may play a role in the degradation of certain SOS-induced proteins that would be deleterious to the cell if they persisted after the completion of the SOS response. This speculation is drawn from a series of observations concerning interactions of the *sulA*, *lon*, and *htpR* genes and gene products. The *sulA* gene product appears to function as an inducible inhibitor of filamentation (112, 146). Cells containing a *lon* mutation exhibit extensive *sulA*$^+$-dependent filamentation after UV irradiation (112, 118); the *lon* gene product seems to regulate the amount of filamentation by affecting the rate of degradation of the *sulA* gene product (234). Since the Lon protein is an adenosine triphosphate-dependent protease (46, 49), it may carry out some or all of this degradation itself, although the results to date do not rule out an indirect role of the Lon protein in this degradation. Another protein that is degraded more slowly in *lon* mutants than in wild-type cells is a mutant sigma subunit of RNA polymerase (123). This mutant sigma subunit is degraded even more slowly in an *htpR* mutant than in a *lon* mutant (C. A. Gross, personal communication), suggesting that directly or indirectly *htpR* may play a role in controlling the activity of the Lon protease and probably at least one other protease. Taken together, these observations suggest the possibility that certain proteases, including the Lon protease, are induced or activated by treatments that induce the heat shock response and that one of their possible roles is to speed the turnover of certain otherwise deleterious SOS-induced proteins, such as SulA, which may have been induced by the same treatment. Consistent with this postulated role for *htpR*-controlled proteases are the observations that higher doses of UV are needed to induce the heat shock system than the SOS system and that induction of the heat shock proteins after UV proceeds more slowly than the induction of SOS proteins (Krueger and Walker, in press).

ADAPTIVE RESPONSE TO METHYLATING AND ETHYLATING AGENTS

Besides the SOS and the heat shock networks, one other independent regulatory network in *E. coli* is known to be induced in response to agents that damage DNA. The existence of this network was first discovered by Samson and Cairns (293) in the course of a study on the effects of continuous exposure of *E. coli* to low concentrations of mutagens. It was observed that if *E. coli* was first exposed to low concentrations of methylating or ethylating agents, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) or ethyl methanesulfonate, it became resistant to the mutagenic and lethal effects of higher doses of these agents. De novo protein synthesis was required, and these physiological responses to methylating and ethylating agents were collectively termed the adaptive response (152). The induced resistance to killing by MNNG was shown genetically to be dependent on polymerase I function, but the induced resistance to MNNG mutagenesis was shown not to be *polA*$^+$-dependent (153, 159).

The adaptive response was shown to be separate from the SOS response since it was not induced by SOS-inducing agents such as UV or 4-nitro-quinoline-1 oxide and could be induced in *recA*(Def) and *lexA*(Ind$^-$) mutants (152). It was,

however, blocked by mutations mapping at a locus termed *ada* (151); mutations termed *adc* which cause the constitutive expression of the adaptive response (317) have also been mapped to the *ada* locus (314). I will refer to the genes induced by methylating and ethylating agents and regulated by the *ada* locus as being members of the adaptive regulatory network.

Biochemistry of the Adaptive Response

To date, two enzymes have been described that are induced as part of the adaptive response and act on DNA damaged by methylating or ethylating agents (192). One, the O^6-alkylguanine-DNA alkyltransferase, seems to play a role primarily in preventing mutagenesis by these agents by removing the potentially mutagenic lesion O^6-alkylguanine. The other, 3-methyladenine-DNA glycosylase II, plays a role in preventing cell killing by these agents and in removing some class of premutagenic lesion other than O^6-alkylguanine. Other enzymes that are induced as part of the adaptive response may well be identified in the future.

O^6-Alkylguanine-DNA alkyltransferase. As mentioned above, the production of O^6-alkylguanine lesions poses a particularly grave mutagenic threat to a cell since such lesions can give rise to mutations by mispairing during replication; the participation of the SOS processing system is not required (78, 82, 176, 208). One of the proteins induced as part of the adaptive response specifically removes this type of lesion. When challenged with a methylating agent such as MNNG, *E. coli* cells induced for the adaptive response accumulate less O^6-methylguanine in their DNA than unadapted cells (38, 39, 310). The development of an in vitro assay for the removal of O^6-methylguanine residues by extracts of adapted cells (103, 156) allowed the demonstration that the repair of an O^6-methylguanine residue in DNA was accomplished by the transfer of the methyl group from the O^6-position of guanine to the cysteine residue of a protein (103, 259). The protein that catalyzes the transfer of the alkyl group from O^6-methylguanine or O^6-ethylguanine residues has been purified and has been shown also to be the protein to which the alkyl group is transferred (72, 193, 316). Consistent with the suggestions from in vivo studies (287), each protein molecule can only act once to remove an alkyl group from an O^6-alkylguanine residue. The semantic issue of whether to call this activity an enzyme has been addressed in some detail elsewhere (192, 193). In this review, I will refer to this activity as an O^6-alkylguanine-DNA alkyltransferase.

O^6-Alkylguanine-DNA alkyltransferase is present in unadapted *E. coli* at a level of approximately 20 to 60 molecules per cell and is present at approximately 100- to 200-fold higher levels in adapted cells or in cells that carry an *adc* mutation (194, 232). The gene coding for this protein has not yet been identified; however, it does not seem to be the product of the *ada* locus. Strains carrying *ada* mutations have the same levels of this activity as unadapted wild-type cells. Furthermore, the 18,000-dalton monomer molecular weight of this protein (72) does not correspond to the molecular weights of either of the *ada* gene products described below.

3-Methyladenine-DNA glycosylase II. The second enzymatic activity reported to be induced as part of the adaptive response is 3-methyladenine-DNA glycosylase II (96, 155). This enzyme has a broad in vitro substrate specificity and, in addition to 3-methyladenine, is able to release 3-methylguanine, 7-methylguanine, and 7-methyladenine from methylated DNA (155). 3-Methyladenine-DNA glycosylase II is the product of the *alkA* gene (96, 162, 375, 376). *E. coli* has a second enzyme, 3-methyladenine-DNA glycosylase I, the product of the *tag* gene, which is only able to release 3-methyladenine (96, 157, 191, 280). The *tag* gene product accounts for 90 to 95% of the 3-methyladenine-DNA glycosylase activity in uninduced cells but, unlike the *alkA* gene product, is not induced as part of the adaptive response (96, 155). 3-Methyladenine-DNA glycosylase II is induced approximately 20-fold during the adaptive response (192). The function of the glycosylases is to initiate excision repair of these methylated bases. The excision repair process requires polymerase I and appears to play a major role in helping cells overcome the lethal effects of exposure to methylating or ethylating agents (10, 153, 376).

Regulation of the Adaptive Network

The regulation of the adaptive network has not yet been studied in as great detail as the SOS network; however, progress is beginning to be made. The *ada* locus appears to code for a positively acting regulatory element(s), since an insertion of a transposon in the *ada* locus to generate an *ada*(Def) mutation results in loss of the ability to induce the adaptive response (P. K. LeMotte and G. C. Walker, unpublished data). The *ada* locus has been cloned and seems to consist of an operon coding for two proteins with molecular weights of 38,000 and 28,000 (315; P. K. LeMotte and G. C. Walker, unpublished data). By constructing an *ada-lac* fusion and by using other techniques, it has been possible to show that the *ada* gene(s) is itself strongly induced in an ada^+-dependent fashion when cells are exposed to a methylating agent such as MNNG (P. K. LeMotte and G. C. Walker, unpublished results). Furthermore, the presence of the *ada* gene(s) on a high-copy-number plasmid leads to elevated expression of O^6-alkylguanine-DNA transferase and 3-methyladenine-DNA glycosylase II in the absence of an inducing treatment (315). It thus seems likely that alkylating agents generate an inducing signal that leads to the induction of the *ada* gene(s) and that it is the higher levels of the *ada* gene(s) that in turn cause the induction of the two repair enzymes.

The nature of the inducing signal for the adaptive response is still unknown. However, considering the specificity of the induction, it seems possible that O^6-alkylguanine adducts may figure in the production of the inducing signal. One obvious possibility is that the alkylated form of the alkyltransferase plays a role in the induction of the *ada* genes. At present, the genes known to be members of the adaptive network are the two *ada* genes themselves, *alkA*, and the as yet unidentified gene coding for the O^6-alkylguanine-DNA alkyltransferase. As the system is studied in more detail, it seems likely that additional genes will be identified as being members of this regulatory network. Recently, the Mu dl-(Ap *lac*) bacteriophage has been used to identify another locus that is induced by methylating agents and is regulated by *ada* (M. R. Volkert, personal communication).

Effect of the Adaptive Response on Mutagenesis

Although methylating and ethylating agents can act as mutagens by introducing lesions (such as O^6-methylguanine) that can cause mutation by directly mispairing, they also introduce lesions that require SOS processing to cause mutations. The nature and relative abundance of the alkylated products that result from exposure of DNA to such agents are influenced by the chemical nature of the individual alkylating agent, particularly by the relative amount of S_N1 and S_N2 character in its alkylating reactions. For example,

agents such as MNNG or *N*-methyl-*N*-nitrosourea produce a greater proportion of O^6-methylguanine adducts than an agent such as methyl methanesulfonate (9). A minor component of MNNG mutagenesis seems to require the participation of SOS processing (309) whereas, as discussed below, most of the mutagenesis caused by methylating agents such as methyl methanesulfonate seems to require SOS processing.

The effect of the O^6-alkylguanine-DNA alkyltransferase on mutagenesis apparently is simply to repair the mispairing lesion before it is replicated. The alkyltransferase repairs O^6-alkylguanine residues in single-stranded DNA much more slowly than in double-stranded DNA, and Lindahl et al. (193) have speculated that this could account for the tendency of agents such as MNNG and *N*-methyl-*N*-nitrosourea to cause multiple mutations at the replication points of bacterial chromosomes. This model is consistent with the observation of Sklar (330) and Sklar and Strauss (331) that chloramphenicol treatment of *E. coli*, a treatment that prevents the induction of the adaptive response (293), enhances MNNG-induced single-site mutations without a proportionate increase in closely linked multiple mutations.

The *polA*$^+$-dependent excision repair process initiated by 3-methyladenine-DNA glycosylase II removes not only potentially lethal lesions but also premutagenic lesions that require SOS processing to cause mutations. These inferences are drawn from the observations that *alkA* mutants, which lack 3-methyladenine-DNA glycosylase II, have an increased sensitivity to mutagenesis by methyl methanesulfonate and that *alkA recA*(Def) double mutants are not mutated by methyl methanesulfonate (376). It is not yet clear whether these premutagenic lesions are the same as, or a subset of, the potentially lethal lesions or whether they are different.

It is possible that at least one of the functions of the constitutive levels of the adaptive response repair enzymes in an uninduced cell may be to remove lesions introduced by the endogenous methylating agent, *S*-adenosylmethionine. Treatment of DNA with *S*-adenosylmethionine leads to the formation of small amounts of 3-methyladenine and 7-methylguanine (8, 254, 290).

Comparison of the SOS, Heat Shock, and Adaptive Regulatory Systems

Our present knowledge of the responses of *E. coli* to agents that damage DNA is summarized in Fig. 4. Three independent regulatory networks can be induced, each with its own set of regulatory control elements. As discussed above, a subset of SOS-inducing agents can induce heat shock genes, although it is not clear that generation of an inducing signal for heat shock involves any type of DNA damage. Methylating agents, such as MNNG, which induce the adaptive response can also induce the SOS responses (3, 273); in fact, the same low dose of MNNG which induces the adaptive response also induces the SOS responses (3). However, as discussed above, the in vivo inducing signals for the SOS and adaptive systems are likely to be quite different. In addition, recent work by Demple and Halbrook (71) has raised the possibility that there may be a fourth regulatory system induced by oxidizing agents and concerned with the repair of oxidative DNA damage.

It is interesting that each of the three systems has a positively acting regulatory element, RecA, HtpR, and Ada, respectively. The SOS system has a negatively acting control element, LexA; however, it has not yet been definitively established whether negatively acting control elements are involved in the regulation of the heat shock and adaptive networks. The Ada element apparently differs from RecA with respect to activation since simply overproducing the Ada product(s) results in the expression of the adaptive responses, whereas overproducing RecA itself does not result in expression of the SOS responses. The RecA and Ada regulatory elements share two other common properties: they are both induced strongly by treatments that induce the systems that they regulate and, in each case, their own function is required for their own induction. In the future, it will be extremely interesting to see (i) how the regulation of these three systems compare at the molecular level and (ii) the number and identity of the genes that are members of each regulatory network.

ROLE OF SOS PROCESSING IN MUTAGENESIS

Mutagenesis By UV and Most Chemicals Is Not a Passive Process

The most fundamental concept that has emerged concerning mutagenesis by UV and many chemicals, for example 4-nitroquinoline-1-oxide and methyl methanesulfonate, is that such mutagenesis is not a passive process. Rather, it requires the intervention of a cellular system that processes damaged DNA in such a way that mutations result. This type of processing has been most often referred to as error-prone repair or SOS repair (276, 371), terms proposed on the basis of a number of observations in *E. coli* that suggest that the cellular events involved in producing mutations from damaged DNA are closely associated with events increasing resistance to killing by chemicals and radiation. However, despite the fundamental significance of such a cellular processing system to chemical and radiation mutagenesis, its biochemical mechanism has not yet been determined nor have the effects on survival and mutagenesis even been rigorously shown to result from the same events. In an effort to avoid implying a particular biochemical mechanism, I will use the relatively neutral term SOS processing to refer to the particular processing of damaged DNA that leads to the introduction of mutations.

The initial evidence suggesting the existence of SOS processing came from physiological and genetic experiments which indicated that some system in *E. coli* had to be induced for mutations to result as a consequence of DNA damage. In retrospect, it was the fact that the SOS processing system in *E. coli* is inducible that first drew attention to its existence. The concept that organisms take an active role in mutating their DNA after exposure to UV radiation or many chemicals is somewhat counterintuitive, and it is possible that the existence of such a system might not have been recognized until much later if it had been constitutively expressed in *E. coli*.

Weigle reactivation and Weigle mutagenesis. The physiological experiments that most clearly suggest the existence of an inducible system required for mutagenesis are those in which UV-irradiated bacteriophage infect UV-irradiated cells. Weigle (363) first noted that preirradiation of *E. coli* cells with a low dose of UV greatly increased the survival of UV-irradiated lambda, a phenomenon later termed Weigle reactivation (276). Furthermore, and very strikingly, UV irradiation of the lambda had very little effect on the phage mutation frequency unless the cells had been preirradiated with UV before phage infection, in which case high amounts of phage mutagenesis were observed, a phenomenon later termed Weigle-mutagenesis. In other words, it seemed as though the UV-induced lesions in the phage DNA did not

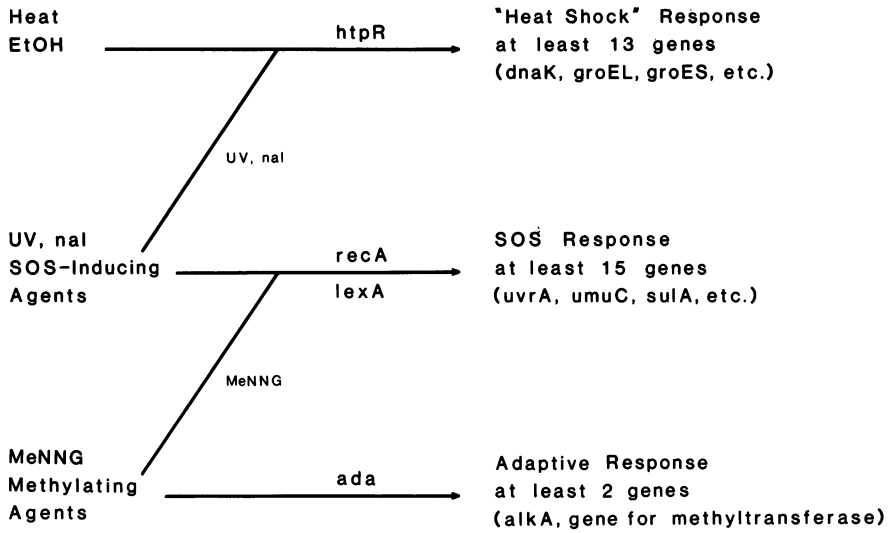

FIG. 4. Three regulatory networks in *E. coli* that can be induced by exposure to DNA-damaging agents (Krueger and Walker, in press).

lead to the production of mutations unless the bacterial host had also been damaged.

Defais et al. (70) later showed that Weigle reactivation and Weigle mutagenesis responses were not observed if the *E. coli* cells carried either *recA*(Def) or *lexA*(Ind$^-$) mutations and proposed that the cellular functions responsible for the increased phage survival and phage mutagenesis were induced in a *recA*$^+$ *lexA*$^+$-dependent fashion by UV irradiation of the bacteria (70).

The suggested inducibility of the system was supported by the observation that the induction of Weigle reactivation and Weigle mutagenesis was inhibited by the presence of chloramphenicol during the period of time after the UV irradiation of the cells but before infection with the UV-irradiated bacteriophage (69). Additional evidence supporting the inducibility came from experiments with strains carrying the *recA441* mutation. If a *recA441* strain was grown under conditions in which the other SOS responses were constitutively expressed, then Weigle reactivation and Weigle mutagenesis were observed even though the bacterial DNA had not been damaged (44). Since some new cellular function(s) apparently had to be induced for UV-induced lesions in the phage DNA to lead to mutations, these results implied the existence of an SOS processing system in *E. coli*.

The power of the Weigle mutagenesis experiments resulted from the fact that they created a situation whereby the existence of SOS processing could be deduced because of its absence in an uninduced cell. Evidently, the basal level of expression of the SOS processing system is quite low in an uninduced cell and, fortuitously, infection with the damaged lambda DNA does not cause a sufficient degree of induction of the SOS system for the SOS processing response to be expressed. Thus, in these Weigle mutagenesis experiments, an uninduced cell is acting in some senses as though it were a mutant defective in the SOS processing system.

Bacterial mutagenesis. A number of experiments suggested that the requirement for processing of UV-irradiated DNA to obtain mutations was not confined to bacteriophage lambda mutagenesis but also applied to chromosomal mutagenesis, and these have been reviewed extensively by Witkin (371). Of particular significance was the observation that UV mutagenesis of the bacterial chromosome was *recA*$^+$ *lexA*$^+$ dependent (175, 233, 252, 368, 371). Thus, the products of genes controlling the induction of a variety of physiological responses, including Weigle reactivation and Weigle mutagenesis, were also required for mutagens such as UV to cause mutations in the chromosome. This result again suggested that some cellular processing system had to be induced for such mutagenesis to occur.

The notion that some cellular system had to be induced for UV mutagenesis of the bacterial chromosome to occur was also supported by experiments carried out by Witkin (369, 370) with *recA441* mutants. A *recA441* strain was irradiated with a UV dose too low to induce the SOS responses, and the amount of bacterial mutagenesis was examined. If the *recA441* strain was incubated at 42°C after the UV irradiation, a condition in which the SOS responses are expressed constitutively, then considerably more mutants were obtained than at 30°C, a condition in which the SOS responses are not expressed.

Significance of *umuD* and *umuC* Mutations

For many years, genetic analysis of the molecular basis of SOS processing was complicated by the fact that at least some of the genes that are required for this process are members of the SOS regulatory network. A number of genes or particular alleles of genes were shown to affect the phenomena, such as Weigle mutagenesis, which suggests the existence of an inducible SOS processing system. Examples of these include (see Table 2) alleles of *recA* such as *recA*(Def) (371), *recA430* (116, 242), *recA441* (44, 369, 370), and *recA453* (45); alleles of *lexA* such as *lexA*(Ind$^-$) (252), *lexA41* (251), and *lexA*(Def) (249); and *ssb* (120). As is obvious in retrospect, the interpretation of the effects of these mutations on SOS processing was extremely complicated since all of them affect the regulation of the entire SOS system as was discussed above. It was not coincidental that all of the mutations originally studied that affected the ability of *E. coli* to be mutated by UV and other agents also affected SOS regulation since all of these mutations were originally identified on the basis of some other phenotype, and their effect on mutagenesis was noted subsequently. Thus, these studies did not lead to the identification of mutations that

specifically affected SOS processing, but rather to the isolation of SOS regulatory mutations.

Isolation of *umuD* and *umuC* mutations. A major advance in our understanding of SOS processing occurred when Kato and Shinoura (166) and, independently, Steinborn (333) directly screened for mutations that abolished the ability of *E. coli* cells to be mutagenized by UV or 4-nitroquinoline-1-oxide. By screening directly for nonmutability, they were able to search for mutants that were defective in SOS processing without necessarily being defective in SOS induction. Three classes of nonmutable mutants were obtained. Mutations in the first class were mapped to the *recA* locus and were shown to resemble the previously isolated *recA430* mutation (116, 166). Mutations in the second class appeared to be alleles of *lexA* (166). Most interestingly, however, mutations in the third class were mapped to a new locus at about 25 min on the *E. coli* chromosome which was termed *umuC*. The mutations at this locus were later shown to lie in two adjacent genes, *umuD* and *umuC* (92, 361). Mutations in either the *umuD* or *umuC* gene cause the same phenotype.

Strains containing a *umuD* or *umuC* mutation are nonmutable with a wide variety of agents including UV, 4-nitroquinoline-1-oxide (166, 333), methyl methanesulfonate (360), and neocarcinostatin (90). They can, however, still be mutated by a few chemical mutagens such as MNNG or ethyl methanesulfonate that produce directly mispairing lesions which do not require SOS processing to cause a mutation (164, 165, 308) and to some extent by the frameshift mutagen ICR 191 (164). They are somewhat sensitive to UV killing and are defective in Weigle reactivation and Weigle mutagenesis of UV-irradiated bacteriophage lambda (166, 334, 360) and of UV-irradiated single-stranded phage (M. Defais, personal communication; L. Marsh and G. C. Walker, unpublished data). However, *umuD* and *umuC* mutants differ from *recA* and *lexA* mutants in that they are still capable of expressing a variety of SOS responses such as induction of lambda prophage (166, 334), filamentous growth (166), and increased synthesis of the RecA protein (360). The simplest interpretation of *umuD* and *umuC* genes based on these phenotypes is that they code for products that are uniquely required for SOS processing.

The original *umuD* and *umuC* mutations isolated by Kato and Shinoura (166) and by Steinborn (333) were derived by MNNG and ethyl methanesulfonate mutagenesis, respectively, so the possibility existed that the nonmutability of the strains was due to the alteration of some function rather than by the loss of a function. However, *umuC* mutants have now been isolated by insertion mutagenesis (3, 92), suggesting that the phenotype of these mutants results from the loss of some cellular function and furthermore that *umuC* is not an essential gene.

The insertion mutation in *umuC* isolated by Bagg et al. (3) was generated by using the Mu dl(Ap *lac*) bacteriophage. Analysis of the regulation of β-galactosidase in *umuC-lac* fusion strains indicated that the *umuC* gene was induced by SOS-inducing treatments and was regulated by the *recA* and *lexA* gene products. Genetic analysis of its regulation was consistent with LexA functioning as a repressor of the gene (3). These observations indicated that at least part of the inducibility of SOS processing is due to the inducibility of the *umuC* gene.

Identification of the *umuD* and *umuC* gene products. The *umuDC* locus has now been cloned and its transcriptional organization determined, and the *umuD* and *umuC* gene products have been identified (92, 164, 326, 359, 361). The *umuD* gene codes for a 16,000-dalton protein and the *umuC* gene codes for a 45,000-dalton protein. The two genes are organized in an operon with the *umuD* gene located upstream of the *umuC* gene. Sequencing studies of the *umuDC* locus have revealed that the final A of the UGA stop codon of the *umuD* gene is the A of the AUG codon that initiates the *umuC* gene (B. B. Mitchell, S. J. Elledge, and G. C. Walker, unpublished results). A variety of genetic studies are consistent with the *umuDC* locus being repressed by the LexA protein (3, 92, 326, 361). Furthermore, as shown in Table 2, two regions with homology to known LexA-binding sequences are located upstream of the *umuDC* locus.

umuD and *umuC* Analogs on Naturally Occurring Plasmids

A number of naturally occurring plasmids (2, 48, 236), for example R46 (83, 247), R205 (212), TP110 (81), and Col I (144, 145), have the ability to make both *E. coli* and *S. typhimurium* more resistant to killing by UV and a number of chemicals and more susceptible to mutagenesis by these agents. As discussed in more detail below, it has been shown recently that at least some of these naturally occurring plasmids carry analogs of the *E. coli umuD* and *umuC* genes (77, 265, 324, 334, 347, 357, 360).

pKM101. A derivative of R46 termed pKM101 has been particularly intensively studied. pKM101 was isolated from R46 by Mortelmans and Stocker (247) and apparently arose by an in vivo deletion of 14 kilobase pairs of DNA which coded for several drug resistances (34, 184, 185). pKM101 is approximately 35 kilobase pairs in size and makes cells somewhat more mutable than does R46 (218), but in other respects the plasmids seem to have very similar properties. Because of its ability to enhance the susceptibility of cells to mutagenesis, pKM101 was introduced in the Ames *S. typhimurium* (218) strains used for the detection of mutagens and carcinogens (Fig. 5) and it has played a major role in the success of the system (216, 217).

E. coli or *S. typhimurium* cells containing pKM101 or R46 exhibit an increased susceptibility to both base substitution

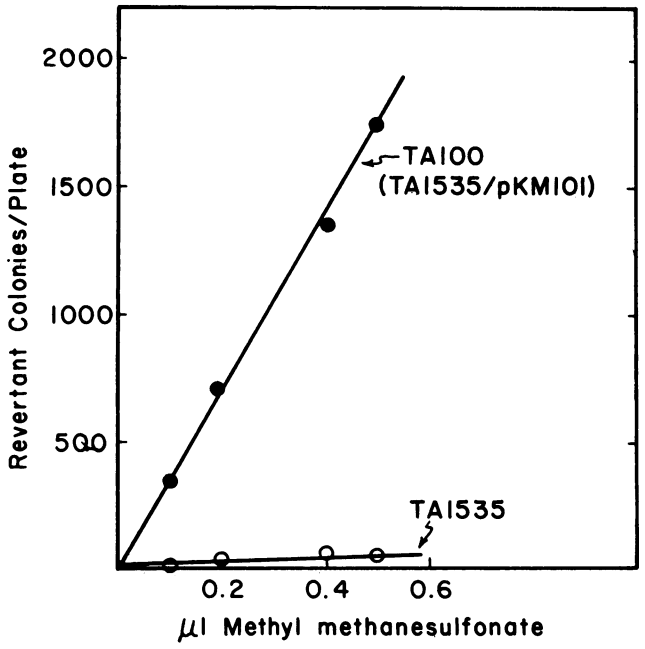

FIG. 5. Effect of the plasmid pKM101 on the reversion of the *S. typhimurium* strain TA1535 with methyl methanesulfonate.

and frameshift mutagenesis with a wide variety of agents (217, 218, 246) as well as an increased resistance to killing by UV (237). Certain potent carcinogens such as aflatoxin B1 and furylfuramide are either nonmutagenic or extremely weakly mutagenic in the absence of pKM101 yet are strongly mutagenic in its presence (218). In addition, pKM101 increases the survival and mutagenesis of UV-irradiated bacteriophage in both unirradiated (354) and irradiated cells (354, 356). The presence of pKM101 in a cell has only a modest influence on the spontaneous mutation frequency (107, 247, 354).

Relationship of pKM101-mediated process to cellular gene functions. pKM101 was shown to increase both susceptibility to mutagenesis and resistance to killing by UV in *uvrA*, *uvrB*, *uvrC*, *polA*, *recB recC*, and *recF* strains (119, 246, 247, 353, 354). In contrast, the ability of pKM101 to influence mutagenesis and repair was shown to be dependent on the *recA*$^+$ *lexA*$^+$ genotype in *E. coli* (119, 237, 246, 353, 354). These observations suggested that pKM101 was interacting in some way with the cellular SOS system. It did not seem likely, however, that pKM101 was acting by causing a general induction of the SOS system since the synthesis of *recA* protein (199, 357) and the stability of lambda lysogens (119) were unaffected by the presence of pKM101. It thus seemed that in some way pKM101 was specifically enhancing the ability of cells to carry out SOS processing.

The key insight into the mode of action of pKM101 and other mutagenesis-enhancing plasmids stemmed from the observation that pKM101 and R46 suppress the nonmutability of *umuD* and *umuC* mutations (Fig. 6) (166, 334, 357, 360). Recently, several other naturally occurring plasmids have also been shown to suppress the nonmutability of *umuC* mutants (347). Although there were a number of formal explanations for this result, the simplest was that pKM101 coded for analogs of the *umuD* and *umuC* functions of *E. coli*.

***mucA* and *mucB* genes of pKM101 are analogs of *umuD* and *umuC*.** It was possible to isolate both MNNG (355) and Tn*5* insertion mutants (324) of pKM101 which had completely lost their ability to influence mutagenesis and repair. Mapping the Tn*5* insertions that caused this phenotype led to the identification of an approximately 2,000-base-pair-long region of pKM101 termed *muc* which was necessary for these plasmid-mediated effects (184, 324). By subcloning the *muc* region (265) and constructing deletion mutants of pKM101 (184), the *muc* region was also shown to be sufficient to increase the susceptibility of cells to mutagenesis and their resistance to killing by UV.

Analysis of the *muc* locus of pKM101 has revealed that it consists of two genes, *mucA* and *mucB*, and that the plasmid-encoded *mucAB* locus is very similar to the chromosomally encoded *umuDC* locus. The *mucA* gene codes for a 16,000-dalton protein and the *mucB* gene codes for a 45,000-dalton protein. The two genes are organized in an operon with the *mucA* gene located upstream of the *mucB* gene. Sequencing studies have revealed that the reading frames of the two genes overlap by 10 base pairs (B. B. Mitchell, K. L. Perry, and G. C. Walker, unpublished data).

A *mucB'-lacZ'* gene fusion was constructed by using in vitro techniques and was used to show that the *mucAB* locus is induced by SOS-inducing treatments and to provide genetic evidence that the operon is repressed by the LexA protein (92). A sequence with homology to known LexA-binding sequences is located upstream of the *mucAB* operon (Table 3). The inducibility of the *mucAB* locus is consistent with the ability of pKM101 to enhance Weigle reactivation of

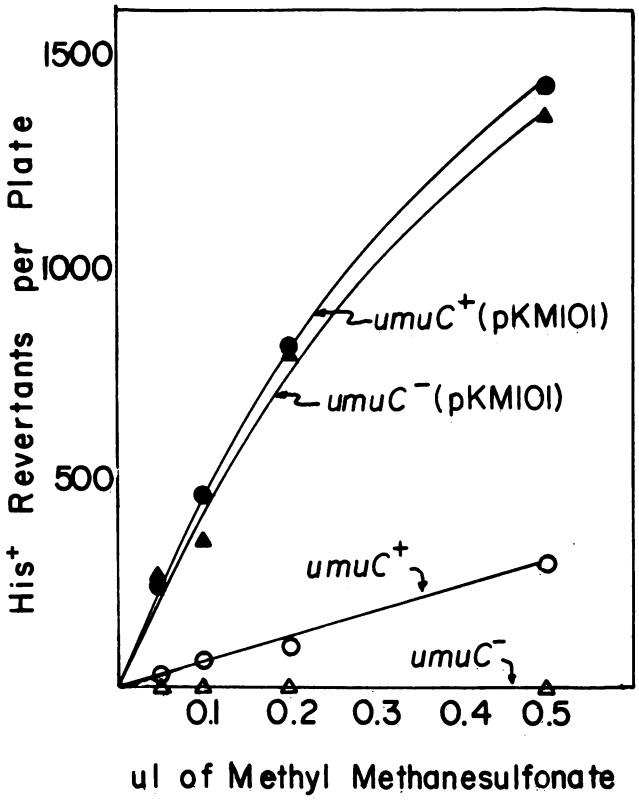

FIG. 6. Suppression of the nonmutability of a *umuC* mutant of *E. coli* by pKM101.

UV-irradiated bacteriophage (354, 356) and with the synergistic interactions that have been observed between pKM101 and the *recA441* mutation (50, 79, 354). It has been suggested that R46, the parent of pKM101, codes for an element which negatively regulates the expression of the *muc* genes (81).

Although the *mucA* and *mucB* gene products seem to be functionally homologous with the *umuD* and *umuC* gene products and the genetic organization of the two loci indicates that they are related, the two loci have apparently undergone considerable evolutionary divergence. The deduced amino acid sequences of the UmuD and MucA proteins are only approximately 35% homologous (B. B. Mitchell, K. L. Perry, S. J. Elledge, and G. C. Walker, unpublished data). The *mucB* and *umuC* genes are currently being sequenced. The degree of difference between the two loci suggests that they diverged a considerable amount of time ago. It is possible, however, that the rate of the evolutionary divergence of the two loci could be accelerated relative to a pair of loci in Umu$^-$ backgrounds since *umuDC* and *mucAB* code for functions which increase the amount of mutagenesis resulting from DNA-damaging treatments (S. J. Elledge, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1983).

Distribution of *umuDC* functions in other bacteria. A number of bacteria such as *Haemophilus influenzae* (173, 257), *Proteus mirabilis* (140), *Methylococcus capsulatus* (134), *Methylobacter* sp. (27), *Deinococcus* (formerly *Micrococcus*) *radiodurans* (338, 341), and *Streptococcus pneumoniae* (111) are nonmutable with UV and in that respect resemble *E. coli umuD* and *umuC* mutants. A variety of observations have suggested that *S. typhimurium* LT2 is deficient in *umuDC* function relative to *E. coli* (358, 359). Consistent

with this hypothesis, the introduction into *S. typhimurium* LT2 of pKM101 (358, 359), an *E. coli* F′ plasmid containing the *umuDC* locus (329), or a recombinant plasmid containing the cloned *umuDC* locus (G. C. Walker and S. J. Elledge, unpublished data) greatly increases its ability to carry out Weigle reactivation of UV-irradiated bacteriophage P22 and its susceptibility to mutagenesis. Similarly, the introduction of R46, the parent of pKM101, into *P. mirabilis* (140) has been shown to result in the bacterium becoming mutable by UV, suggesting that *P. mirabilis* is normally nonmutable because it lacks *umuDC* function. Whether other bacteria are UV nonmutable for the same reason remains to be determined. It is interesting, however, that an analog of *umuDC* is found on a relatively broad host range plasmid, and that the *mucAB* locus of pKM101 is surrounded by inverted repeats (184), suggesting that it might be, or might once have been, part of a transposable element.

The two strains in the Ames test that are in general the most sensitive and that detect the largest number of compounds are the pKM101-containing *S. typhimurium* strains TA100 and TA98 (216–218). Since *S. typhimurium* LT2 seems to be deficient in *umuDC* function and since pKM101 provides functions analogous to *umuDC*, it seems likely that any compound that is more mutagenic in strain TA100 or TA98 than in the corresponding strains lacking the plasmids (TA1535 and TA1538, respectively) is dependent on *umuDC* function, in whole or in part, for its mutagenic effect. In other words, any such compound requires SOS processing to be mutagenic.

A Second Requirement for the RecA Protein in Mutagenesis

It is now clear that the RecA protein plays some other role in the process of UV and chemical mutagenesis besides being required for the induction of the *umuDC* genes. This can most easily be seen by the fact that *lexA*(Def) *recA* strains are nonmutable with UV (3, 11, 202) despite the fact that the *umuD* and *umuC* genes are constitutively expressed at high levels in such backgrounds (3). From a priori considerations, this second requirement for RecA function for mutagenesis could be due to the RecA protein carrying out a second regulatory function besides cleaving *lexA* repressor, the RecA protein playing a mechanistic role in the process, or the RecA protein carrying out both a second regulatory function and a mechanistic function. The issue will obviously not be settled in an unambiguous fashion until the complete biochemistry of mutagenesis has been worked out in vitro. Several experiments have been performed, however, which shed light on this question.

Effect of the *recA430* mutation on mutagenesis. More detailed insights into the nature of the requirements for RecA function can be gained by consideration of the effects of the *recA430* allele on mutagenesis. Strains carrying this allele are proficient for homologous recombination (12). Consistent with this, the RecA430 protein has a nearly normal adenosine triphosphatase activity and catalyzes strand exchange in vitro (281). As discussed above, however, it appears to have a reduced ability to cleave the LexA protein.

Since strains carrying a *recA430* mutation probably do not induce the *umuDC* genes to a very great extent, it is not too surprising that such strains are poorly mutated by UV (12, 116, 242). *lexA*(Def) *recA430* strains, however, are also only weakly mutated by UV in spite of the fact that they express the *umuDC* genes constitutively at high levels because of the absence of functional LexA protein (11). A *lexA*::Tn*5*(Def) *recA430* strain in which the LexA protein has been completely eliminated (178) has the same phenotype so that the effect is not due to incomplete derepression of the *umuDC* genes. The simplest explanations for this observation are that (i) the proteolytic capability of the RecA protein is required for the process of mutagenesis to occur because it cleaves the repressor of some gene that is not repressed by the LexA protein; (ii) the proteolytic capability of the RecA protein is required because it cleaves some protein which then participates in the actual biochemical process of mutagenesis; and (iii) some characteristic of the RecA protein which is needed to activate its proteolytic capabilities, for example the ability to bind to a particular substrate, is also required for mutagenesis, but the protease activity per se is not required other than to induce *umuDC*.

Evidence suggesting that UV mutagenesis does not require the induction of genes other than those repressed by LexA. Evelyn Witkin (personal communication) has recently performed an experiment which suggests that RecA is not needed for the induction of any gene(s) besides those repressed by the LexA protein. A *lexA*(Def) *recA441* strain was grown at 30°C in rich medium containing guanosine and cytidine, conditions known to prevent the activation of the proteolytic capabilities of the RecA441 protein. The culture was then washed and given a dose of UV irradiation too low to cause effective induction of the SOS response. If the irradiated cells were then plated immediately on rich medium containing guanosine plus cytidine, no mutagenesis was observed. However, if the irradiated cells were incubated for 1 h at 42°C in minimal medium containing adenine, conditions known to lead to an activation of the RecA441 proteolytic capabilities, and then plated on rich medium containing guanosine plus cytidine, mutants were obtained. Significantly, the same number of mutants were obtained if the incubation at 42°C was carried out in the presence of chloramphenicol.

In this experiment, the *umuDC* genes were expressed constitutively because of the *lexA*(Def) mutation in the strains. For UV mutagenesis to occur, however, the cells still required conditions leading to the activation of the RecA protease, suggesting that the RecA protein has a second role in mutagenesis besides inducing the *umuDC* genes. Furthermore, this experiment suggests that the role of the activated RecA protein is not to induce another gene since the presence of chloramphenicol during the period of RecA activation did not affect the number of mutants obtained. If the interpretation of this experiment is correct, the result would eliminate the first possibility listed above.

Other evidence. A few other observations suggest that the ability of the RecA protein which allows it to be proteolytically activated is important for the second role of RecA in mutagenesis. By themselves they do not indicate whether the protease activity is actually required or whether it is simply the ability of RecA to be activated which is important. For example, a *lexA*(Def) *recA441* strain has an elevated spontaneous mutation rate which is *umuDC* dependent (11). The ability of *recA441* to increase the spontaneous mutation rate may be a reflection of its ability to become proteolytically active in undamaged cells and the possible basis of such mutagenesis is discussed in the following section. The spontaneous mutation rate of this strain is increased by conditions which activate the RecA441 protease activity (11). Similar observations have been made by Witkin et al. with the *recA730* allele (373).

If the amount of RecA protein in a *lexA*(Def) *recA441* strain is kept low by introducing a putative promoter-down

mutation, *recA453*, the high level of spontaneous mutagenesis is reduced (11). Thus, even if the *umuDC* genes are fully expressed, RecA is required in substantial amounts for the *umuDC*-dependent increase in spontaneous mutation rate to be observed. The *lexA*(Def) *recA441 recA453* mutant is reasonably mutable by UV and is, in fact, more mutable than the *lexA*(Def) *recA430* strain (11). This result indicates that, in the case of UV mutagenesis, a low amount of RecA that can be activated is more effective for mutagenesis than a larger amount of RecA that has a defect in becoming proteolytically activated.

Similarly, a *lexA*(Def) *recA441* strain exhibits an increased susceptibility to mutagenesis by the *umuDC*-dependent mutagen methyl methanesulfonate under conditions which activate the *recA441* protease (J. H. Krueger and G. C. Walker, unpublished data). The effect may be observable with this compound and not so readily with UV since methyl methanesulfonate is a relatively weak SOS-inducing agent (273) and therefore poor at causing events which lead to the activation of RecA.

Targeting of UV and Chemical Mutagenesis

The realization that cellular functions are required for mutagenesis raised the issue as to whether mutations introduced by UV and chemical mutagenesis are targeted or untargeted. In other words, does the induced SOS processing system introduce mutations specifically at the sites of lesions in DNA or does it introduce mutations at random sites regardless of whether there are lesions at those sites or not? It is now clear that most of the mutations that arise after the treatment of *E. coli* with UV or chemical mutagens are the result of a targeted cellular process. This topic has recently been reviewed by Miller (228). However, it does appear likely that, at least in certain circumstances and at lower frequency, untargeted mutagenesis can occur as a consequence of the action of a processing system on DNA that has not been damaged by an exogenous agent.

Before discussing the evidence supporting these statements it seems necessary to define some terminology. First, from a priori considerations, simply classifying mutations as to whether they arose from a targeted or untargeted process is inadequate since there are two ways in which a mutagenic process could be targeted. Such a process could be locally targeted (the presence of the lesion leads to the introduction of mutations at its actual site) or it could be regionally targeted (the presence of the lesion leads to the introduction of mutations in its immediate vicinity but not directly at its site). Second, lesions such as apurinic sites or cyclobutane pyrimidine dimers have often been referred to as being "noninformational," "noninstructional," "nonpairing," or "noncoding" because their chemical structure differs greatly from the normal structures found in DNA; however, the basis of this classification also seems inadequate. A true noninformational lesion would be one that does not play a role itself in influencing the nature of the sequence alteration resulting from the presence of the lesion. However, lesions that differ even grossly from normal structures found in DNA could, through a combination of hydrogen-bonding, van der Waals interactions, and stacking interactions, still favor the preferential incorporation of a particular nucleotide or base at the site of the lesion; I will refer to such lesions as being pseudoinformational. It is probably worth noting that in principle both noninformational and pseudoinformational lesions could either result directly from a chemical interaction of an agent with DNA or they could be second-order lesions arising from the interaction of the cell with the original chemical lesion.

Most UV and chemical mutagenesis is targeted. The insight that most UV and chemical mutagenesis is targeted was made possible by the use of experimental approaches that allowed mutations to be characterized with regard to the nucleotide change(s) they cause. The first system that allowed large numbers of mutations to be studied conveniently at this level was the fine-structure *lacI* genetic system of *E. coli* developed by J. Miller and his colleagues (59, 231). With this system, a set of purely genetic techniques can be used to analyze amber and ochre mutations occurring within the *lacI* gene in such a way that the sequence changes giving rise to these mutations can be determined; all possible base substitution mutations can be detected except for adenine (A) · thymine (T) → guanine (G) · cytosine (C). A variety of other types of systems have also been employed to distinguish genetically between various types of mutations (5, 100, 107, 167, 302, 305, 379). An alternative type of approach which has been utilized more widely recently has been to select or screen for mutations occurring on bacteriophage or plasmids and then use DNA sequencing techniques to determine directly the sequence changes that caused these mutations (109, 188, 204, 235; R. D. Wood and F. Hutchinson, J. Mol. Biol., in press).

Sets of *lacI* nonsense mutations caused by several different SOS-dependent mutagens have been analyzed. A comparison of the types and frequencies of mutations caused by UV (59, 344), 4-nitroquinoline-1-oxide (59), neocarcinostatin (104), (±)7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10 tetrahydrobenzo[*a*]pyrene (BPDE) (89), 3,4-epoxycyclopenta[*c,d*] pyrene (89), activated aflatoxin B1 (106), and *cis*-diaminedichloroplatinum (II) (33) clearly indicates that each agent causes its own characteristic spectrum of mutations. Thus, most of the base substitution mutations caused by these agents must be targeted for, otherwise the same spectrum of mutations would have been generated by all of the mutagens (105, 228). More specifically, this means that the particular lesions generated as a consequence of exposure to these various agents must play a role in determining the outcome of the mutations that result. As will be discussed below, it is possible that a small fraction of the mutations could result from an untargeted process.

An independent line of evidence arguing that most UV mutagenesis is targeted comes from experiments showing that UV-irradiated phage plated on UV-irradiated cells have a much higher mutation frequency than unirradiated phage plated on the same cells (Weigle mutagenesis) (70, 363); this effect is *umuDC* dependent (166, 272). Furthermore, the types and locations of mutations observed with the irradiated phage are different from those observed with the unirradiated phage (Wood and Hutchinson, in press). Thus, the extra mutations observed when the phage have been irradiated must result from targeted mutagenesis carried out by an SOS processing system since the host cells have been equivalently induced in both cases. The relative importance of untargeted mutagenesis in these experiments is discussed below.

Hotspots. It is clear that the local DNA sequence plays a role in determining the efficiency with which a particular mutagen causes mutations at particular sites. For example, in the case of UV mutagenesis, some sites (hotspots) are very much more mutable than others (59). The location of the hotspots observed depends on the mutagen used, so hotspots are not due to there being regions of DNA which are intrinsically more mutable by all agents than other regions of DNA. The DNA sequence surrounding a poten-

tially mutable site could play a role in determining the frequency of mutation by affecting (i) the relative frequency or nature of the lesions that occur at that site or both (i.e., by influencing the chemistry or photochemistry that occurs at that site), (ii) the efficiency of accurate repair systems in removing lesions from that site, or (iii) the efficiency with which SOS processing can occur at that damaged site.

In the case of UV mutagenesis, Todd and Glickman (344) have reported that a Δ*uvrB* strain which lacks the ability to carry out excision repair of pyrimidine dimers and other bulky lesions had, with one exception, the same spectrum of mutational hotspots as was observed with a *uvrB*$^+$ strain. This result indicates that for most of the hotspots the local sequence does not affect the efficiency of accurate repair but rather the efficiency with which lesions are introduced or the efficiency with which SOS processing occurs at the damaged sites or both. The one exceptional site that was influenced by *uvr*$^+$ functions lay in the terminal loop of a quasi-palindromic sequence, and it was suggested that in this case the effect of the local sequence was to influence the secondary structure of the DNA in the immediate region of the site; at least one effect of the secondary structure might be to influence the efficiency of a *uvr*$^+$-dependent accurate repair process (344). This suggestion is consistent with some of the unusual effects that have been noted in studies of mutagenesis at suppressor loci (15, 28, 260, 299, 371). Glickman (115) has also reported that, although the presence of pKM101 increased the susceptibility of both *uvr*$^+$ and *uvrB* mutants to UV mutagenesis, the mutational spectra of *lacI* mutations are nearly identical in the presence and absence of pKM101. Eisenstadt et al. (89) have obtained similar results with two polycyclic aromatic mutagens. These observations reinforce the conclusion that the *mucA* and *mucB* genes are functional analogs of the *umuD* and *umuC* genes.

Concept of HFOs and LFOs. Although the observation that different agents cause different spectra of base substitution mutations indicates very strongly that most of the mutagenesis observed after treatment of *E. coli* with UV or a chemical mutagen is targeted, it does not in and of itself indicate whether such mutagenesis is locally or regionally targeted. Foster et al. (105) have further analyzed the frequencies of different mutations resulting from treatment with UV, 4-nitroquinoline-1-oxide, and neocarcinostatin and have concluded that such mutations arise from two classes of events: nonrandom, high-frequency occurrences (HFOs) and apparently random, low-frequency occurrences (LFOs).

(i) HFOs. The base pair changes giving rise to HFO mutations are different for different mutagens. This suggests that the chemical nature of the lesions resulting from exposure to the various agents plays a major role in determining the nucleotide which is ultimately incorporated. Certainly the simplest model to account for this result is that the HFOs arise from a locally targeted process in which a nucleotide is incorporated directly opposite a pseudoinformational lesion. HFO events do not occur with equal frequency at all sites where they can be observed. As discussed above, the efficiency with which a given site can be mutated seems to depend on the chemical nature of the mutagen and the DNA sequence surrounding the site.

(ii) LFOs. In contrast, the base pair changes giving rise to the LFOs appear to be random, with all possible base changes being observed. In spite of this apparent randomness with respect to base pair change, Foster et al. (105) conclude that the LFO mutations are also targeted since a number of sites in the *lacI* gene that gave rise to LFOs with UV and 4-nitroquinoline-1-oxide fail to give rise to LFOs with neocarcinostatin. If the LFOs arose by an untargeted process then one would expect the same set of LFOs to be produced with each mutagen. The two simplest ways to explain the LFO mutations are that they arise (i) from a locally targeted process taking place at relatively rare noninformational lesions or (ii) from a regionally targeted process occurring in the vicinity of lesions.

The most concentrated set of probable LFOs obtained to date would seem to be the set of mutations obtained by Youderian et al. (378) in their analysis of the bacteriophage P22 promoter P_{ant}. The phage were mutated by infecting UV-irradiated P22 bacteriophage into UV-irradiated pKM101-containing cells. Mutations were observed at every base pair in the −10 and −35 regions of the promoter, and all changes were observed except for G · C → C · G. The absence of G · C → C · G transversions is probably not significant since the regions consist mostly of A · T bases and a base pair change was detected only if it affected promoter function. Since no particular site or base pair change dominated the spectrum of mutations obtained, these regions do not appear to contain a hotspot for UV mutagenesis, and the mutations do not seem to be HFOs. Since the cells were maximally induced for SOS processing and the increase in mutation frequency required UV irradiation of the bacteriophage, these mutations would appear to correspond to the LFO events of Foster et al. (105).

Consequences of SOS Processing of DNA Not Damaged by Exogenous Agents

In spite of the fact that most UV and chemical mutagenesis appears to be targeted to sites of damage on DNA, there are two types of experiments which suggest that the SOS processing system can cause mutagenesis by operating on DNA which has not been damaged by an exogenous agent. The first type is experiments in which the SOS processing system of *E. coli* is induced/activated by genetic means, and an increased rate of spontaneous mutation is observed even though the cellular DNA has not been deliberately damaged. The second type is experiments in which undamaged bacteriophage are introduced into SOS-induced cells and an increased rate of phage mutagenesis is induced. In principle, mutations arising from the action of SOS processing on DNA not damaged by an exogenous agent could result from (i) untargeted mutagenesis by the SOS processing system or (ii) targeted mutagenesis resulting from the processing of "cryptic" lesions (370, 371) which are innocuous or lethal unless the SOS processing system is induced. For example, such cryptic lesions could, in principle, be produced by fluorescent light (371), spontaneous depurinations, damage by endogenous alkylating agents, damage by oxygen radicals, etc.

Relationship of SOS processing to the spontaneous mutation frequency. Experiments in which the SOS processing system is induced by genetic means have usually utilized strains carrying the *recA441* allele. Growing a *recA441* strain at 42°C in the presence of adenine, a condition that results in the induction of many of the SOS responses, causes a large increase in the spontaneous reversion rate of *trp* ochre mutation in *E. coli* B/r (369, 370) and of the *his-4* and *argE3* ochre mutations in *E. coli* K-12 (50, 112, 354). The increase in the spontaneous mutation rate seen in *recA441* strains is *umuDC*$^+$-dependent (50), and the introduction of pKM101 into the *recA441* strains led to an even larger increase in the spontaneous mutation rate at 42°C (50, 79, 354). These observations indicate that the increase in spontaneous mutagenesis results from the *umuDC*-dependent SOS processing

system acting on DNA that has not been damaged by an external agent. It seems unlikely that the growth at 42°C is introducing premutagenic lesions since another *recA* allele, *recA730*, which causes induction of SOS functions even at 30°C, also causes an increase in the spontaneous mutation rate at 30°C, similar to that seen in a *recA441* mutant at 42°C (373). Moreover, it appears that the effect of the higher temperature and adenine is not simply to induce the *umuDC* genes since the effect is also seen with *lexA*(Def) *recA441* strains as discussed above in detail.

Although shifting a *recA441* mutant to 42°C leads to a large increase in the spontaneous reversion frequency of the alleles listed above, the reversion frequency of a number of other base-substitution mutations is not affected or is affected very little (A. Schauer and G. C. Walker, unpublished data). These observations suggest that there are hotspots for this type of untargeted mutagenesis. This conclusion is consistent with a number of observations indicating that the introduction of a plasmid such as pKM101 into a cell increases the spontaneous reversion frequency of certain mutations but not of others (107, 246). If one of the effects of pKM101 is to increase the basal level of processing activity in cells (354), then the increased spontaneous mutation frequency could be due to the SOS processing system interacting with DNA in an undamaged cell. The hotspots for this type of mutagenesis could be the result of a preference of the SOS system to produce untargeted mutations at particular DNA sequences or it could reflect the distribution of cryptic lesions in undamaged DNA.

The mutations produced by growing a *recA441* strain under conditions where the SOS responses are expressed have recently been analyzed by using the *lacI* system (J. H. Miller and K. B. Low, personal communication). The frequency of spontaneous mutations observed was elevated but was much lower than the frequencies observed after UV or chemical treatment, consistent with the notion that most UV and chemical mutagenesis is targeted. A considerable increase in G · C → T · A transversions at a number of sites was observed along with some increase in A · T → T · A transversions at other sites. Some hotspots were observed along with some coldspots. Whether a base pair was a hotspot or not seemed to be totally determined by the flanking sequences. These observations are consistent either with SOS processing causing untargeted mutagenesis preferentially at specific sequences or with there being hotspots for mutagenesis resulting from cryptic lesions.

Sargentini and Smith (300) have recently carried out a detailed analysis of the effect of various repair mutations on the spontaneous mutation rate under different growth conditions. They have concluded that (i) since *umuC*, *recA*(Def), or *lexA*(Ind$^-$) mutations lower the spontaneous mutation rate, a component of spontaneous mutagenesis results from the action of the basal level of SOS processing activity that is present in uninduced cells and (ii) since inactivation of nucleotide excision repair by *uvrA* or *uvrB* mutation raises the spontaneous mutation rate, at least some spontaneous mutations are the result of lesions in undamaged cells being acted on by the basal level of SOS processing.

Mutagenesis of undamaged bacteriophage in SOS-induced cells which depends on SOS processing. Other experiments, which suggest that mutagenesis can occur by the action of the SOS processing system on DNA which has not been damaged with an exogenous agent, involve infecting UV-irradiated cells with undamaged lambda bacteriophage. Quillardet and Devoret (272) have shown that an undamaged λ *vir2 vir3* bacteriophage mutates to virulence at a higher frequency when plated on UV-irradiated cells than when plated on unirradiated cells. This increase in the mutation rate of undamaged phage brought about by the UV preirradiation of the bacterial host was not seen if the cells carried a mutation at the *umuDC* locus.

Mutagenesis of undamaged bacteriophage in SOS-induced cells which does not depend on SOS processing. It now seems evident that at least one other mechanism can contribute to phage mutagenesis in such circumstances. Several groups have carried out analogous experiments in which an increase in the frequency of clear plaque mutants of lambda was detected when unirradiated bacteriophage were plated on UV-irradiated host cells (30, 147, 272; Wood and Hutchinson, in press). The fact that this phenomenon was not observed if the host carried a *recA* mutation (147), provided an early suggestion that the mutations arose from untargeted mutagenesis by the error-prone processing system. However, in analyzing this further, Wood and Hutchinson (in press) have found that (i) increased phage mutagenesis is seen if the host carries both *recA* and *recB* mutations, (ii) increased phage mutagenesis is seen even if the host carries a *umuC36* mutation, and (iii) the mutations that result consist mainly of frameshift mutations occurring at runs of identical bases. Taken together, these results suggest that the mutations were not generated by the action of an SOS processing system. Since the effect seems to be enhanced in a *polAex1* mutant and since cells with a low level of polymerase I have been shown to have an increased spontaneous mutation frequency due to increased frameshift mutagenesis, Wood and Hutchinson (in press) have suggested that frameshift mutations arise during an attempt to replicate DNA in the absence of adequate levels of polymerase I. They envision the UV-induced lesions in the bacterial chromosome as tightly binding much of the polymerase I of the cell so that less than normal would be available for bacteriophage replication. A delay in joining Okazaki fragments would then increase the likelihood of a frameshift mutation occurring by slippage as suggested by Streisinger et al. (336).

The differing results obtained by Quillardet and Devoret (272) and Wood and Hutchinson (in press) can be rationalized by considering the different systems used in the two experiments to detect mutations. For virulent mutants to arise, a mutation had to occur within the OR_2 region of the *c*I operator, whereas clear plaque mutants could arise by a wide variety of mutations within the *c*I gene. Although the SOS processing system could have been responsible for some of the *c*I mutants obtained, the majority arose by some process which is *umuDC* independent. Two factors could have contributed to the *umuDC* dependence of the virulent mutations: (i) the OR_2 region does not contain any runs of identical bases longer than two and therefore might be expected to be a poor region for frameshift mutants to occur in and (ii) the OR_2 region could be a hotspot for the SOS processing system for one of the reasons discussed above.

Even though relatively high levels of untargeted mutagenesis have been reported when infecting undamaged lambda phage into UV-irradiated cells and measuring the frequency of *c*I mutants (30, 272), caution must be exercised when using these numbers to deduce the ratio of targeted to untargeted mutagenesis in experiments where the infecting phage have been damaged. The clear plaques detected when the phage have been damaged result from bursts of phage containing only mutant phage (Wood and Hutchinson, in press), indicating that the fixation of the mutation occurred before completion of the first round of replication on the damaged template or, alternatively, that a mutation was

fixed every time the damaged template was replicated. In contrast, the clear plaques detected when the phage have not been damaged result from bursts containing mostly wild-type phage mixed with one or a few mutant phage (272; Wood and Hutchinson, in press), indicating that the mutation occurred late in infection, probably during the rolling circle mode. Thus, the actual mutation frequency is considerably less than the observed frequency of clear plaques, a situation which has led to overestimates of the relative importance of the contribution of untargeted mutagenesis to the mutagenesis induced by UV.

The results discussed in this section suggest that although the SOS processing system in *E. coli* can cause mutations operating on DNA that has not been damaged by an exogenous agent, it produces mutations with far greater efficiency when operating on DNA that contains suitable lesions such as those introduced by UV. Mutations arising with DNA that has not been damaged by an exogenous agent may come from SOS processing of naturally occurring lesions or simply from untargeted mutagenesis brought about by the SOS processing system acting on regions of DNA that do not contain lesions; some evidence favors the notion that naturally occurring lesions are involved. Furthermore, at least one other process favoring frameshift mutations can contribute to mutagenesis in undamaged DNA in certain situations.

What Constitutes a Premutagenic Lesion for SOS Processing?

Given that most UV and chemical mutagenesis is targeted and that at least a substantial fraction of such mutagenesis appears to result from the action of the SOS processing system directly at the site of pseudoinformational or noninformational lesions, the determination of the chemical structure of such premutagenic lesions becomes a matter of considerable interest. A final rigorous proof that a particular lesion constitutes a premutagenic lesion must probably await the synthesis of DNA molecules containing that lesion at a single known site. Nevertheless, a considerable amount of evidence has been accumulated suggesting that two lesions, AP (apurinic or apyrimidinic) sites and the UV-induced pyrimidine-pyrimidine (6-4) photoproduct (135) serve as premutagenic lesions for the SOS processing system.

Apurinic sites. A series of experiments carried out by Loeb and his colleagues strongly suggest that apurinic sites are premutagenic lesions which when acted on by the SOS processing system lead to the production of mutations. The basic observation is that single-stranded φX174 DNA which has been exposed to conditions which cause depurination is mutated if it is transfected into SOS-induced spheroplasts but not if it is transformed into uninduced spheroplasts (306). The lesion causing the mutagenesis was shown to be an apurinic site by demonstrating that treatment of the depurinated phage template with either alkali (306) or an apurinic endonuclease from HeLa cells (306) prevented the mutagenesis. The process requires the involvement of SOS processing since it is *umuC*$^+$- and *recA*$^+$-dependent (304). For the effect to be observed, the spheroplasts must also be *recF*$^+$; however, the role of *recF* in the process is unclear. The RecF protein could be required for efficient induction of the SOS system after UV irradiation or it could actually play a role in the SOS processing of single-stranded bacteriophage as suggested by Clark and Volkert (53).

It is possible that apurinic sites may play a role in the mutagenicity of a number of chemical carcinogens. Schaaper et al. (303) have presented data consistent with the notion that mutagenesis by β-propiolactone proceeds by the generation of apurinic sites by depurination of alkylated bases. In addition, Foster et al. (106) have suggested that mutagenesis by aflatoxin B1, BPDE, and 3,4-epoxycyclopenta[*c,d*]pyrene also proceeds by the generation of apurinic sites from damaged bases. Such depurination could proceed spontaneously since alkylation at some positions such as N(7) of guanine labilizes the glycosidic bond or it could require the action of a glycosylase.

Pyrimidine-pyrimidine (6-4) photoproduct. The nature of the UV-induced photoproduct which is responsible for UV mutagenesis has been a matter of great interest for many years (371). Study of this problem has been greatly complicated, however, by the fact that UV induces a number of different photoproducts, that more than one photoproduct can occur at the same site in different DNA molecules, that these various photoproducts are susceptible to a number of types of accurate DNA repair, that SOS processing is required for UV-induced lesions to give rise to mutations, and by the fact that UV is an efficient inducer of both certain of the accurate repair systems and of the SOS processing system. Nevertheless, circumstantial evidence is accumulating which tends to suggest that a UV-induced photoproduct, the pyrimidine-pyrimidine (6-4) lesion (108, 135, 195), is at least one of the important premutagenic lesions involved in UV mutagenesis.

The evidence supporting the possible involvement of pyrimidine-pyrimidine (6-4) lesions in UV mutagenesis consists of two types of observations. The first is that the location of UV-induced base substitution mutations seems to correlate better with sites of expected pyrimidine-pyrimidine (6-4) formation than with the sites of expected pyrimidine cyclobutane dimer formation (23; R. D. Wood, T. R. Skopek, and F. Hutchinson, J. Mol. Biol., in press). In one case, the frequency of pyrimidine-pyrimidine (6-4) and pyrimidine cyclobutane dimer lesions was measured directly in a fragment of the *lacI* gene, and positions which were hotspots for the formation of pyrimidine-pyrimidine (6-4) lesions were shown to correlate with the locations of hotspots for UV mutagenesis (23). The second type of observation is that introduction of photoproducts with 313-nm radiation and the sensitizer α-aminoacetophenone leads to a different spectrum of mutations from those that are observed upon standard 254-nm UV irradiation (Wood et al., in press). This treatment leads to the formation of T-T and some T-C cyclobutane dimers but not to the formation of pyrimidine-pyrimidine (6-4) product, suggesting that, at least at some sites, the pyrimidine-pyrimidine (6-4) lesion is more efficient at causing a mutation than a cyclobutane pyrimidine dimer.

What Is the Biochemical Mechanism of SOS Processing?

A number of models have been proposed over the years for the mechanism of SOS processing, but its biochemistry is still unknown at the time that this review is being written. However, given the fact that at least some of the gene products required for this process have been identified and are presently being purified and studied in a number of laboratories, there would seem to be a reasonable chance that the actual biochemical mechanism will be determined in the relatively near future.

Likelihood that a polymerase plays a role in SOS processing. The work reviewed above indicates that UV and much chemical mutagenesis results from the induced SOS processing system introducing an incorrect base into a damaged DNA molecule. An incorrect base could be introduced into a DNA molecule by a step in which a new phosphodiester

bond is formed at the time of introduction of the incorrect base, and most models for the biochemical mechanism of SOS processing have postulated the involvement of a new or modified polymerase which introduces an incorrect nucleotide (18, 29, 88, 164, 277, 305, 335, 371). Alternatively, an incorrect base could, in principle, be introduced by mechanisms which do not require the formation of a new phosphodiester bond at the site of the incorrect base. For example, a short oligonucleotide with homology to the region containing the lesion could be "grafted" in by a recombinational event (92) or an incorrect base could be introduced by an "insertase" (205) which introduces a base rather than a nucleotide.

In spite of these formal alternatives, it seems most likely that the mechanism of SOS processing will turn out to involve a modified or even a new polymerase. It is difficult to reconcile the specificity of mutagenesis with regard to base change and site that is seen for different mutagens with the introduction of incorrect bases by a recombinational event. An insertase mechanism could account for the specificity of base pair changes in a double-strand DNA molecule but not in a single-strand molecule. The one report of an insertase activity in *E. coli* (205) has not been substantiated by work in a second laboratory (161). In contrast, introduction of an incorrect base by a polymerase is (i) compatible with the notion that the properties of a lesion can influence the nature of the base-pair change that results and (ii) compatible with the observation that SOS processing can apparently act on both single- and double-strand damaged DNA templates. Most models proposed to account for SOS processing assume that the polymerase involved is inserting deoxyribonucleotides although it has recently been suggested that, for bacteriophage T4, bypass of dimers might occur by the synthesis of primer RNAs opposite the site of the lesion (64).

Action of DNA polymerases on damaged DNA templates. The action of known DNA polymerases on damaged DNA templates has been studied by a variety of different systems (17, 18, 180, 181, 238–241, 266, 305, 335). The exact result depends somewhat on the polymerase being used; however, it seems clear that DNA damage introduced by agents such as UV, *N*-acetoxyacetylaminofluorene, and dimethyl sulfate blocks the progress of normal polymerases. Polymerization terminates just before or at the site of the lesion. In some cases if the stringency of the polymerase is relaxed, for example by adding Mn^{2+}, additional nucleotides can be incorporated, suggesting that it is possible for less stringent polymerases to operate on such damaged templates (238, 240, 335). In general, mammalian cell DNA polymerases, which at least as presently isolated lack proofreading activity, are able to bypass at least some of these lesions (17, 18, 180, 238, 266, 305) and this has led to the suggestion that an inhibition of the 3′-5′ proofreading function of a known polymerase might account for the mechanism of SOS processing.

Loeb and his colleagues (180, 181, 305) have carried out model experiments demonstrating that the bypass of an apurinic site, an apparent premutagenic lesion for SOS processing, by a polymerase can lead to the introduction of mutations. Both they and Strauss et al. (275, 335) have pointed out that the preference of a polymerase for inserting a particular nucleotide or a particular class of nucleotide, such as a purine nucleotide, could influence the mutagenic consequences of a polymerase replicating across a noninformational lesion.

Physiological relevance. The experiments in which DNA polymerases introduce mutations by bypassing lesions on damaged DNA templates demonstrate the feasibility of SOS processing occurring by such a mechanism. It has not yet been shown, however, whether such in vitro reactions with known DNA polymerases have any physiological relevance to the process by which UV and chemical mutagenesis occurs in living cells. The three gene products which are known to play physiologically relevant roles in this process are UmuD, UmuC, and RecA, and the final biochemical model for the mechanism of mutagenesis by SOS processing will have to explain the roles of these three gene products satisfactorily. Until such a mechanism is established, a considerable number of formal possibilities remain. For example, if the UmuD and UmuC gene products were to act by leading to the formation of a polymerase with a capability of acting on damaged templates, they could do so by directly coding for such an activity themselves, by modifying an existing polymerase, or by regulating the induction of yet another protein which then participates directly in the biochemical mechanism.

The problem is illustrated by the recent report by Lackey et al. (182) of the isolation of an altered form of polymerase I from SOS-induced cells. The fidelity of this new polymerase activity is much less than that of polymerase I on undamaged DNA templates. This polymerase could conceivably play a role in SOS processing; however the fact that it can be isolated from *umuC* mutants (D. Lackey and S. Linn, personal communication) leaves the issue in doubt. The polymerase might play no role at all in SOS processing or it could be that it does play a role and that UmuC plays yet some other role. A similar problem exists with establishing whether SOS-induced RecA-dependent immortal replication (174, 186) bears any relationship to SOS processing. Although it is SOS induced and requires the participation of RecA, it can still be observed in *umuC* mutants (S. J. Elledge, K. L. Perry, and G. C. Walker, unpublished data), so that its relevance to mutagenesis is not clear.

It has taken the combined and varied efforts of many labs over many years to advance our knowledge to a point where it should be realistically possible to determine the biochemistry of this fundamental cellular process required for UV and chemical mutagenesis and it will be extremely interesting to learn how it proceeds.

EFFECTS OF OTHER REPAIR SYSTEMS ON MUTAGENESIS

The efficiency with which a lesion can give rise to a mutation is also influenced by repair systems in *E. coli* which carry out accurate repair. This area has recently been summarized by Hartman (133).

***uvr*$^+$-dependent excision repair.** The *uvrA*, *-B*, and *-C* genes of *E. coli* code for a complex endonuclease (295, 321, 322, 377) which initiates excision repair of bulky lesions such as UV-induced pyrimidine dimers by incising both 5′ and 3′ of the lesion (295). The mechanism of this type of repair has recently been reviewed (122, 132, 135, 192). Most *uvr*$^+$-dependent excision repair produces a relatively short patch and must be very accurate since agents such as UV are far more mutagenic in *uvr* mutants. A second type of *uvr*$^+$-dependent repair termed long-patch repair also appears to be accurate since it is still observed in *umuC* mutants (57). The fact that the *uvrA*, *-B*, and *-C* genes seem to be members of the SOS regulatory network means that treatments which induce the SOS system increase the capacity of a cell to carry out this type of accurate repair. Part of the Weigle reactivation of UV-irradiated lambda which is seen in *uvr*$^+$ cells seems to be due to an increased capacity of the cells to

carry out excision repair.

Excision repair initiated by a glycosylase and an AP endonuclease. Another major strategy used by *E. coli* and other organisms to initiate excision repair is to use glycosylases to excise specific damaged or incorrect bases by cleaving their glycosylic bonds and then an AP endonuclease to cleave the DNA at the AP sites that are generated. This subject has recently been reviewed in depth by Lindahl (192). As discussed above, 3-methyladenine-DNA glycosylase II is induced as part of the adaptive response, and it will be interesting to see whether other glycosylases as well as perhaps AP endonucleases will be induced in response to various types of DNA damage.

As with *uvr*$^+$-dependent excision repair, this type of excision repair seems accurate since its main effect seems to be to reduce the number of mutations that arise after a DNA-damaging treatment. An interesting aspect to this type of repair system, however, is raised by the accumulating evidence that AP sites may serve as premutagenic lesions for SOS processing. Since the removal of a damaged or incorrect base by a glycosylase creates an AP site, the subsequent repair events must be very efficient relative to SOS processing. It is possible that circumstances will be identified where the action of a glycosylase increases the amount of SOS-dependent mutagenesis that is observed.

Daughter strand gap repair. It is not clear that the set of recombinational mechanisms which aid in the tolerance of daughter strand gaps (132) plays any role in the introduction of mutations since this type of repair is observed in *umuC* mutants (163). Furthermore, as discussed above, it is difficult to account for the specificity of mutagenesis with different compounds by invoking a recombinational mechanism.

Methyl-directed mismatch repair. An increasing amount of evidence has been accumulating suggesting that *E. coli* possesses a mismatch repair system which is able to repair mismatched base pairs occurring at the replication fork (271, 278). The system requires the products of the *mutH*, *-L*, and *-S*, and *uvrD* genes. Apparently the presence of N^6-adenine methylation at GATC sequences in a parental strand allows it to be discriminated from the newly synthesized (unmethylated) daughter strand; the adenine methylation at GATC sequences is carried out by the product of the *dam* gene (214). Recently, Lu et al. (211) reported the detection of methyl-directed mismatch repair in vitro. As discussed above, the *uvrD* gene is part of the SOS regulatory network but it is not yet clear whether the *mutH*, *-L*, and *-S* genes are regulated.

Two ways have been suggested by which methyl-directed mismatch repair could influence the amount of mutagenesis that results from treatment of cells with a DNA damaging agent. Caillet-Fauquet and Maenhaut-Michel (37, 213) have pointed out that such a mismatch repair system could correct mistakes introduced by what I have termed untargeted and regionally targeted mutagenesis by the SOS processing system and have presented experiments with *mutH*, *-L*, and *-S* mutants which are consistent with this suggestion.

Although mismatch repair can apparently act at O^6-methylguanine · thymine base pairs in *E. coli*, it does not lead to a reduction in the numbers of mutations (158). Shanabruch et al. (323) have discussed a model to explain how mismatch repair could interact with lower levels of O^6-alkylguanine-DNA alkyltransferase activity such as those found in *S. typhimurium* (128) to influence the number of mutations obtained after treatment with a methylating or ethylating agent.

MUTAGENESIS AND INDUCIBLE RESPONSES TO DNA DAMAGE IN OTHER ORGANISMS

As outlined in this review, the organism whose responses to DNA damage we presently understand in the greatest detail is *E. coli*. It will be extremely interesting in the future to see how the strategies employed by other organisms in responding to DNA damage resemble those of *E. coli*.

SOS-like responses. A number of bacteria have been shown to induce physiological responses or new proteins or both in response to agents such as UV which induce the SOS response in *E. coli*. The list of such bacteria includes gram-negative bacteria such as *S. typhimurium*, *P. mirabilis* (138, 139), *H. influenzae* (257), *Bacteriodes fragilis* (311), and *Rhizobium meliloti* (V. Chang, J. H. Krueger, and G. C. Walker, unpublished data) and gram-positive bacteria such as *Bacillus subtilis* (98). Some of these bacteria appear to have SOS systems which are quite closely related to that of *E. coli*. For example, *S. typhimurium* appears to have a LexA protein with virtually the same binding specificity as that of *E. coli*, since the *S. typhimurium uvrD* gene is regulated by LexA in *E. coli* and *E. coli din-lac* fusions are inducible by UV if introduced into *S. typhimurium* (263). The RecA protein of *P. mirabilis* is apparently quite closely related to the *E. coli* RecA protein since it can carry out both the recombinational and SOS-inducing functions of RecA if introduced into *E. coli* (91). The RecE protein of *B. subtilis* appears to have functional similarities to the *E. coli* RecA protein and seems to regulate its own induction by UV (75).

Eucaryotic organisms also seem to have responses which are induced by the same agents which introduce the SOS system in *E. coli*. For example, by the use of *lac* gene fusions constructed in vitro, it has been possible to show that *Saccharomyces cerevisiae* has a set of genes which are induced in response to DNA-damaging treatments (J. W. Szostak and S. W. Ruby, personal communication). Moreover, a number of observations described in the literature indicate that mammalian cells exhibit inducible responses to DNA-damaging agents (36, 97, 168, 183, 187, 298). The p53 cellular tumor antigen of mouse cells can be induced by treating nontransformed mouse cells with UV or a UV-mimetic agent (W. Maltzman and L. Czyzk, personal communication). Examination of the protein profiles on two-dimensional gels of a mammalian cell line treated with BPDE has revealed a subset of proteins induced against a background of changes due to cells ceasing to grow (M. E. Lambert, J. Garrels, I. B. Weinstein, personal communication). The possible existence of such inducible regulatory networks should probably be kept in mind while considering the mode of action of chemical carcinogens. If such a regulatory system were present in mammals, then exposure of an animal to such an agent would not only damage DNA and possibly cause mutations but also cause the induction of new genes with functions not necessarily related to DNA repair or mutagenesis or the induction of viruses.

Heat shock responses. The heat shock response seems to have been conserved in evolution across the procaryote-eucaryote boundary. In fact, the *E. coli* DnaK protein is approximately 50% homologous to the *Drosophila* Hsp70 gene product, which in turn is homologous to a mammalian heat shock protein and to a family of yeast gene products (60). A wide variety of types of stress are known to induce the heat shock response in other organisms so that it is possible that certain DNA-damaging agents might be able to induce the heat shock response in organisms besides *E. coli*.

Adaptive-like responses. The most characteristic activity of

the adaptive response, the O^6-alkylguanine-DNA alkyltransferase, has been identified in a number of different bacteria, including *S. typhimurium* (128), *B. subtilis* (129, 245), and *Micrococcus luteus* (S. Riazzuddin, quoted in reference 192). An adaptive response has been described in *B. subtilis* which is extremely similar to that observed in *E. coli* (129, 245). Interestingly, *S. typhimurium* LT2 does not exhibit an adaptive response even though it synthesizes low constitutive levels of O^6-alkylguanine-DNA alkyltransferase. Since the introduction of cloned *E. coli ada* genes into *S. typhimurium* greatly increases its resistance to both the mutagenic and killing effect of MNNG (P. K. LeMotte and G. C. Walker, unpublished data), it seems possible that *S. typhimurium* LT2 may be a naturally occurring *ada* mutant.

An O^6-alkylguanine-DNA alkyltransferase activity has also been described in mammalian cells (16, 223, 264), and it appears to be inducible in certain cell lines (339, 352). The classification of cell lines as Mer^+ or Mer^- seems to reflect, at least in part, the levels of O^6-alkylguanine-DNA alkyltransferase (68, 95, 332, 352). In some respects, the differences between Mer^+ cells and Mer^- cells are similar to the differences between *E. coli* and *S. typhimurium* in their responses to alkylating agents. In addition, an adaptive response to methylating agents has been observed at a physiological level in certain lines of mammalian cells. Pretreatment of certain lines of Chinese hamster ovary cells and human skin fibroblasts with low levels of MNNG induced resistance to the mutagenic, cytotoxic, and sister chromatid exchange-inducing effects of MNNG (154, 294, 313). It appears that the inducibility of O^6-alkylguanine-DNA alkyltransferase and other related repair enzymes varies from species to species and from cell line to cell line.

Mutagenesis and SOS processing. Considerations of the mechanisms of chemical mutagenesis in eucaryotes have become particularly relevant in light of the recent demonstrations that particular oncogenes differ by a single base pair change from their homologs in normal cells. Perhaps the most important implication of the isolation of *umuD* and *umuC* mutations in *E. coli* is that mutagenesis by UV and other agents requires the active participation of cellular functions. The existence of bacteria which seem to lack an SOS processing system and hence are nonmutable has been described above. Since the cells of many eucaryotic organisms, including humans, can be mutated by such agents, there would seem to be a conceptual need to postulate the participation of an analog of an SOS processing system in these cells. Whether the processing required for mutagenesis in eucaryotic cells is biochemically analogous to that which takes place in *E. coli* cells is a question which one can probably not begin to address until the mechanism of SOS processing in *E. coli* is established.

Efforts to establish the presence of SOS-like processing systems in other organisms have tended to focus on establishing the inducibility of such a system. Results such as to those of Sarasin et al. (21, 298) suggest that such a system may exist in mammalian cells. However, it should probably be kept in mind in future work that the existence or nonexistence of an SOS-like processing system for mutagenesis is a separate issue from the regulation of such a system. One can imagine an organism in which an SOS-like processing system would be constitutively expressed. Inducibility of an SOS-like processing system can be a great help in the experimental study of such a system, but it is not an essential part of the concept that UV and chemical mutagenesis require the active participation of cellular functions.

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LITERATURE CITED

1. **Armengod, M.-E.** 1982. *recF*-dependent recombination as an SOS function. Biochimie **64**:629–632.
2. **Babudri, N., and C. Monti-Bragadin.** 1977. Restoration of mutability in non-mutable *Escherichia coli* carrying different plasmids. Mol. Gen. Genet. **155**:287–290.
3. **Bagg, A., C. J. Kenyon, and G. C. Walker.** 1981. Inducibility of a gene product required for UV and chemical mutagenesis in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:5749–5753.
4. **Bailone, A., A. Levine, and R. Devoret.** 1979. Inactivation of prophage lambda repressor *in vivo*. J. Mol. Biol. **131**:553–572.
5. **Balbinder, E., D. Kerry, and C. I. Reich.** 1983. Deletion induction in bacteria. I. The role of mutagens and cellular error-prone repair. Mutat. Res. **112**:147–168.
6. **Baluch, J., J. W. Chase, and R. Sussman.** 1980. Synthesis of *recA* protein and induction of bacteriophage lambda in single-strand deoxyribonucleic acid-binding protein mutants of *Escherichia coli*. J. Bacteriol. **144**:489–498.
7. **Baluch, J., R. Sussman, and J. Resnick.** 1980. Induction of prophage lambda without amplification of *recA* protein. Mol. Gen. Genet. **178**:317–323.
8. **Barrows, L. R., and P. N. Magee.** 1982. Nonenzymatic methylation of DNA by *S*-adenosylmethionine *in vitro*. Carcinogenesis **3**:349–351.
9. **Beranek, D. T., C. C. Weis, and D. H. Swenson.** 1980. A comprehensive quantitative analysis of methylated and ethylated DNA using high pressure liquid chromatography. Carcinogenesis **1**:595–606.
10. **Billen, D., and G. R. Hellermann.** 1979. Enhancement of deoxyribonucleic acid DNA polymerase I-directed repair synthesis in toluene-tested *Escherichia coli* after growth in the presence of low levels of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. J. Bacteriol. **137**:1439–1442.
11. **Blanco, M., G. Herrera, P. Collado, J. Rebollo, and L. M. Botella.** 1982. Influence of *RecA* protein on induced mutagenesis. Biochimie **64**:633–636.
12. **Blanco, M., A. Levine, and R. Devoret.** 1975. *lexB*: a new gene governing radiation sensitivity and lysogenic induction in *Escherichia coli* K12, p. 379–382. *In* P. Hanawalt and R. B. Setlow (ed.), Molecular mechanisms for the repair of DNA. Plenum Publishing Corp., New York.
13. **Blanco, M., and L. Pomes.** 1977. Prophage induction in *Escherichia coli* K12 cells deficient in DNA polymerase I. Mol. Gen. Genet. **154**:287–292.
14. **Bockrath, R. C., and P. C. Hanawalt.** 1980. Ultraviolet light induction of *recA* protein in a *recB uvrB* mutant of *Escherichia coli*. J. Bacteriol. **143**:1025–1028.
15. **Bockrath, R. C., and J. E. Palmer.** 1977. Differential repair of premutational UV-lesions at tRNA genes in *E. coli*. Mol. Gen. Genet. **156**:133–140.
16. **Bogden, J. M., A. Eastman, and E. Bresnick.** 1981. A system in mouse liver for the repair of O^6-methylguanine lesions in methylated DNA. Nucleic Acids Res. **9**:3089–3103.
17. **Boiteux, S., and J. Laval.** 1982. Mutagenesis by alkylating agents: coding properties for DNA polymerase of poly (dC) template containing 3-methylcytosine. Biochimie **64**:637–641.
18. **Boiteux, S., G. Villani, S. Spadari, F. Zambrano, and M.**

Radman. 1978. Making and correcting errors in DNA synthesis: *in vitro* studies of mutagenesis, p. 73–84. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), DNA repair mechanisms. Academic Press, Inc., New York.

19. **Borek, E., and A. Ryan.** 1958. The transfer of irradiation-elicited induction in a lysogenic organism. Proc. Natl. Acad. Sci. U.S.A. **44**:374–377.
20. **Borek, E., and A. Ryan.** 1973. Lysogenic induction. Prog. Nucleic Acid Res. Mol. Biol. **13**:249–300.
21. **Bourre, F., and A. Sarasin.** 1983. Targeted mutagenesis of SV40 DNA induced by UV light. Nature (London) **305**:68–70.
22. **Brandsma, J. A., D. Bosch, C. Backendorf, and P. van de Putte.** 1983. A common regulatory region shared by divergently transcribed genes of the *Escherichia coli* SOS system. Nature (London) **305**:243–245.
23. **Brash, D. E., and W. A. Haseltine.** 1982. UV-induced mutation hotspots occur at DNA damage hotspots. Nature (London) **298**:189–192.
24. **Brent, R.** 1982. Regulation and autoregulation by *lexA* protein. Biochimie **64**:565–569.
25. **Brent, R., and M. Ptashne.** 1980. The *lexA* gene product represses its own promoter. Proc. Natl. Acad. Sci. U.S.A. **77**:1932–1936.
26. **Brent, R., and M. Ptashne.** 1981. Mechanism of action of the *lexA* gene product. Proc. Natl. Acad. Sci. U.S.A. **78**:4204–4208.
27. **Bridges, B. A.** 1976. Mutation induction, p. 7–14. *In* K. D. MacDonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, Inc., New York.
28. **Bridges, B. A., R. E. Dennis, and R. J. Munson.** 1967. Differential induction and repair of ultraviolet damage leading to true reversions and external suppressor mutations of an ochre codon in *Escherichia coli* B/r WP2. Genetics **57**:897–908.
29. **Bridges, B. A., and R. P. Mottershead.** 1978. Mutagenic DNA repair in *Escherichia coli*. VIII. Involvement of DNA polymerase III in constitutive and inducible mutagenic repair after ultraviolet and gamma irradiation. Mol. Gen. Genet. **162**:35–41.
30. **Bridges, B. A., and M. Southworth.** 1982. Constitutive error-prone repair at sites of excision repair in *Escherichia coli*: a re-examination. Biochimie **64**:655–659.
31. **Bridges, B. A., M. W. Southworth, and E. Orr.** 1983. Mutagenic repair in *Escherichia coli*. VII. Effect of *gyrB* mutations on ultraviolet light mutagenesis. Mutat. Res. **112**:3–16.
32. **Brooks, K., and A. J. Clark.** 1967. Behavior of lambda bacteriophage in a recombination-deficient strain of *Escherichia coli*. J. Virol. **1**:283–293.
33. **Brouwer, J., P. van de Putte, A. M. J. Fichtinger-Schepman, and J. Reedijk.** 1981. Base-pair substitution hotspots in GAG and GCG nucleotide sequences in *Escherichia coli* K-12 induced by *cis*-diaminedichloroplatinum (II). Proc. Natl. Acad. Sci. U.S.A. **78**:7010–7014.
34. **Brown, A. M. C., and N. S. Willetts.** 1981. A physical and genetic map of the *incN* plasmid R46. Plasmid **5**:188–201.
35. **Brozmanova, J., T. P. Iljina, A. S. Saenko, and M. Sedliakova.** 1981. Inducible repair of DNA double strand breaks in *Escherichia coli*. Stud. Biophys. **85**:195–201.
36. **Bullock, P., and M. Botchan.** 1982. Molecular events in the excision of SV40 DNA from the chromosomes of cultured mammalian cells. *In* R. T. Schimke (ed.), Gene amplification. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
37. **Caillet-Fauquet, P., and G. Maenhaut-Michel.** 1982. Increased detection of the SOS mutator effect in *Escherichia coli* mutants deficient in mismatch correction. Biochimie **64**:661–663.
38. **Cairns, J.** 1980. Efficiency of the adaptive response of *Escherichia coli* to alkylating agents. Nature (London) **286**:176–178.
39. **Cairns, J., P. Robins, B. Sedgwick, and P. Talmud.** 1981. The inducible repair of alkylated DNA. Prog. Nucleic Acid Res. Mol. Biol. **26**:237–244.
40. **Capage, M. A., and J. R. Scott.** 1983. SOS induction by P1 Km miniplasmids. J. Bacteriol. **155**:473–480.
41. **Casadaban, M. J., and S. N. Cohen.** 1980. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. U.S.A. **76**:4530–4533.
42. **Casaregola, S., R. D'Ari, and O. Huisman.** 1982. Quantitative evaluation of *recA* gene expression in *Escherichia coli*. Mol. Gen. Genet. **185**:430–439.
43. **Casaregola, S., R. D'Ari, and O. Huisman.** 1982. Role of DNA replication in the induction and turn-off of the SOS response in *Escherichia coli*. Mol. Gen. Genet. **185**:440–444.
44. **Castellazzi, M., J. George, and G. Buttin.** 1972. Prophage induction and cell division in *E. coli*. I. Further characterization of the thermosensitive mutation *tif-1* whose expression mimics the effects of UV irradiation. Mol. Gen. Genet. **119**:139–152.
45. **Castellazzi, M., J. George, and G. Buttin.** 1972. Prophage induction and cell division in *E. coli*. II. Linked (*recA zab*) and unlinked (*lexA*) suppressors of *tif-1*-mediated induction and filamentation. Mol. Gen. Genet. **119**:153–174.
46. **Charette, M. F., G. H. Henderson, and A. Markovitz.** 1981. ATP hydrolysis-dependent protease activity of the *lon*(*capR*) protein of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **78**:4728–4732.
47. **Chase, J. W., J. B. Murphy, R. F. Whittier, E. Lorenson, and J. J. Sninsky.** 1983. Amplification of *ssb-1* mutant single-stranded DNA-binding protein in *Escherichia coli*. J. Mol. Biol. **164**:193–211.
48. **Chernin, L. S., and V. S. Mikoyan.** 1981. Effects of plasmids on chromosome metabolism in bacteria. Plasmid **6**:119–140.
49. **Chung, C. H., and A. L. Goldberg.** 1981. The product of the *lon*(*capR*) gene in *Escherichia coli* is the ATP-dependent protease, protease La. Proc. Natl. Acad. Sci. U.S.A. **78**:4931–4935.
50. **Ciesla, Z.** 1982. Plasmid pKM101-mediated mutagenesis in *Escherichia coli* is inducible. Mol. Gen. Genet. **186**:298–300.
51. **Clark, A. J.** 1973. Recombination deficient mutants of *E. coli* and other bacteria. Annu. Rev. Genet. **7**:67–86.
52. **Clark, A. J.** 1982. *recA* operator mutations and their usefulness. Biochimie **64**:669–675.
53. **Clark, A. J., and M. R. Volkert.** 1978. A new classification of pathways repairing pyrimidine dimer damage in DNA, p. 57–72. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), DNA repair mechanisms. Academic Press, Inc., New York.
54. **Cohen, S., B. J. Knoll, J. W. Little, and D. W. Mount.** 1981. Preferential cleavage of phage lambda repressor monomers by *recA* protease. Nature (London) **294**:182–184.
55. **Cohen, S. P., J. Resnick, and R. Sussman.** 1983. Interaction of single-strand binding protein and *recA* protein at the single-stranded DNA site. J. Mol. Biol. **167**:901–909.
56. **Cole, S. T.** 1983. Characterization of the promoter for the LexA regulated *sulA* gene of *Escherichia coli*. Mol. Gen. Genet. **189**:400–404.
57. **Cooper, P.** 1981. Inducible excision repair in *Escherichia coli*, p. 139–146. *In* E. Seeberg and K. Kleppe (ed.), Chromosome damage and repair. Plenum Publishing Corp., New York.
58. **Cooper, P.** 1982. Characterization of long patch excision repair of DNA in ultraviolet-irradiated *Escherichia coli*: an inducible function under *rec-lex* control. Mol. Gen. Genet. **185**:189–197.
59. **Coulondre, C., and J. H. Miller.** 1977. Genetic studies of the *lac* repressor. IV. Mutagenic specificity in the *lacI* gene of *Escherichia coli*. J. Mol. Biol. **117**:577–606.
60. **Craig, E., T. Ingolia, M. Slater, L. Manseau, and J. Bardwell.** 1982. *Drosophila*, yeast and *E. coli* genes related to *Drosophila* heat-shock genes. *In* M. Schlesinger, M. Ashburner, and A. Tissieres (ed.), Heat shock. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
61. **Craig, N. L., and J. W. Roberts.** 1980. *E. coli recA* protein-directed cleavage of phage lambda repressor requires polynucleotide. Nature (London) **283**:26–30.
62. **Craig, N. L., and J. W. Roberts.** 1981. Function of nucleoside triphosphate and polynucleotide in *Escherichia coli recA* protein-directed cleavage of phage lambda repressor. J. Biol. Chem. **256**:8039–8044.
63. **Crowl, R. M., R. P. Boyce, and H. Echols.** 1981. Repressor

cleavage as a prophage induction mechanism: hypersensitivity of a mutant lambda cI protein to RecA-mediated proteolysis. J. Mol. Biol. **152**:815–819.
64. **Cupido, M.** 1983. Bypass of pyrimidine dimers in DNA of bacteriophage T4 via induction of primer RNA. Mutat. Res. **109**:1–11.
65. **D'Ari, R., J. George, and O. Huisman.** 1979. Suppression of *tif*-mediated induction of SOS functions in *Escherichia coli* by an altered *dnaB* protein. J. Bacteriol. **140**:381–387.
66. **D'Ari, R., and O. Huisman.** 1982. DNA replication and indirect induction of the SOS response in *Escherichia coli*. Biochimie **64**:623–627.
67. **Day, R. S.** 1977. UV-induced alleviation of K-specific restriction of bacteriophage lambda. J. Virol. **21**:1249–1251.
68. **Day, R. S., III, C. H. J. Ziolkowski, D. A. Scudiero, S. A. Meyer, A. S. Lubiniecki, A. J. Giradi, S. M. Galloway, and G. D. Bynum.** 1980. Defective repair of alkylated DNA by human tumour and SV40-transformed human cell strains. Nature (London) **288**:724–727.
69. **Defais, M., P. Caillet-Fauquet, M. S. Fox, and M. Radman.** 1976. Induction kinetics of mutagenic DNA repair activity in *E. coli* following ultraviolet irradiation. Mol. Gen. Genet. **148**:125–130.
70. **Defais, M., P. Fauquet, M. Radman, and M. Errera.** 1971. Ultraviolet reactivation and ultraviolet mutagenesis of lambda in different genetic systems. Virology **43**:495–503.
71. **Demple, B., and J. Halbrook.** 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. Nature (London) **304**:466–468.
72. **Demple, B., A. Jacobsson, M. Olsson, P. Robins, and T. Lindahl.** 1982. Repair of alkylated DNA in *Escherichia coli*. J. Biol. Chem. **257**:13776–13780.
73. **Devoret, R., and J. George.** 1967. Induction indirecte du prophage lambda par le rayonnement ultraviolet. Mutat. Res. **4**:713–714.
74. **Devoret, R., M. Pierre, and P. L. Moreau.** 1983. Prophage ϕ80 is induced in *Escherichia coli* K12 *recA430*. Mol. Gen. Genet. **189**:199–206.
75. **de Vos, W. M., and G. Venema.** 1983. Transformation of *Bacillus subtilis* competent cells: identification and regulation of the *recE* gene product. Mol. Gen. Genet. **190**:56–64.
76. **Dharmalingam, K., and E. B. Goldberg.** 1980. Restriction *in vivo*. V. Induction of SOS functions in *Escherichia coli* by restricted T4 phage DNA, and alleviation of restriction by SOS functions. Mol. Gen. Genet. **178**:51–58.
77. **Dobson, P. P., and G. C. Walker.** 1980. Plasmid(pKM101)-mediated Weigle reactivation in *Escherichia coli* K12 and *Salmonella typhimurium* LT2: genetic dependence, kinetics of induction, and effect of chloramphenicol. Mutat. Res. **71**:25–41.
78. **Dodson, L. A., R. S. Foote, S. Mitra, and W. E. Masker.** 1982. Mutagenesis of bacteriophage T7 *in vitro* by incorporation of O^6-methylguanine during DNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **79**:7440–7444.
79. **Doubleday, O. P., M. H. L. Green, and B. A. Bridges.** 1977. Spontaneous and ultraviolet-induced mutation in *Escherichia coli*: interaction between plasmid and *tif-1* mutator effects. J. Gen. Microbiol. **101**:163–166.
80. **Dowden, S. B., L. Cossins, J. Glazebrook, M. Ramsden, and P. Strike.** 1982. DNA damage inducible genes on the I group plasmid TP110. Biochimie **64**:681–685.
81. **Dowden, S. B., and P. Strike.** 1982. R46-derived recombinant plasmids affecting DNA repair and mutation in *E. coli*. Mol. Gen. Genet. **186**:140–144.
82. **Drake, J. W., and R. H. Baltz.** 1976. The biochemistry of mutagenesis. Annu. Rev. Biochem. **45**:11–37.
83. **Drabble, W. T., and B. A. D. Stocker.** 1968. R (transmissible drug-resistance) factors in *Salmonella typhimurium*: pattern of transduction of phage P22 and ultraviolet-protection effect. J. Gen. Microbiol. **53**:109–123.
84. **Ebina, Y., F. Kishi, T. Miki, H. Kagamiyama, T. Nakazawa, and A. Nakazawa.** 1981. The nucleotide sequence surrounding the promoter region of colicin E1 gene. Gene **15**:119–126.
85. **Ebina, Y., F. Kishi, and A. Nakazawa.** 1982. Direct participation of *lexA* protein in repression of colicin E1 synthesis. J. Bacteriol. **150**:1479–1481.
86. **Ebina, Y., Y. Takahara, F. Kishi, A. Nakazawa, and R. Brent.** 1983. LexA protein is a repressor of the colicin E1 gene. J. Biol. Chem. **258**:13258–13269.
87. **Echols, H.** 1981. SOS functions, cancer and inducible evolution. Cell **25**:1–2.
88. **Echols, H.** 1982. Mutation rate: some biological and biochemical considerations. Biochimie **64**:571–575.
89. **Eisenstadt, E., A. J. Warren, J. Porter, D. Atkins, and J. H. Miller.** 1982. Carcinogenic epoxides of benzo(*a*)pyrene and cyclopenta(*cd*)pyrene induce base substitutions via specific transversions. Proc. Natl. Acad. Sci. U.S.A. **79**:1945–1949.
90. **Eisenstadt, E., M. Wolf, and I. H. Goldberg.** 1980. Mutagenesis by neocarzinostatin in *Escherichia coli* and *Salmonella typhimurium*: requirement for *umuC*$^+$ or plasmid pKM101. J. Bacteriol. **144**:656–660.
91. **Eitner, G., B. Adler, V. A. Lanzov, and J. Hofemeister.** 1982. Interspecies *recA* protein substitution in *Escherichia coli* and *Proteus mirabilis*. Mol. Gen. Genet. **185**:481–486.
92. **Elledge, S. J., and G. C. Walker.** 1983. Proteins required for ultraviolet light and chemical mutagenesis: identification of the products of the *umuC* locus of *Escherichia coli*. J. Mol. Biol. **164**:175–192.
93. **Elledge, S. J., and G. C. Walker.** 1983. The *muc* genes of pKM101 are induced by DNA damage. J. Bacteriol. **155**:1306–1315.
94. **Emmerson, P. T., and S. C. West.** 1977. Identification of protein X of *Escherichia coli* as the *recA*$^+$/*tif*$^+$ gene product. Mol. Gen. Genet. **155**:77–85.
95. **Erickson, L. C., G. Laurent, N. A. Sharkey, and K. W. Kohn.** 1980. DNA cross-linking and monoadduct repair in nitrosourea-treated human tumour cells. Nature (London) **288**:727–729.
96. **Evenson, G., and E. Seeberg.** 1982. Adaptation to alkylation resistance involves the induction of a DNA glycosylase. Nature (London) **296**:773–775.
97. **Fernandez, A., S. Mondal, and C. Heidelberger.** 1980. Probabilistic view of the transformation of cultured C3H/10T 1/2 mouse embryo fibroblasts by 3-methylcholanthrene. Proc. Natl. Acad. Sci. U.S.A. **77**:7272–7276.
98. **Fields, P. I., and R. E. Yasbin.** 1983. DNA repair in *B. subtilis*: an inducible dimer specific W-reactivation system. Mol. Gen. Genet. **190**:475–480.
99. **Fishel, R. A., A. A. James, and R. Kolodner.** 1981. *recA*-independent general genetic recombination of plasmids. Nature (London) **294**:184–186.
100. **Fix, D., and R. Bockrath.** 1983. Targeted mutation at cytosine-containing pyrimidine dimers: studies of *E. coli* B/r with acetophenone and 313 nm light. Proc. Natl. Acad. Sci. U.S.A. **80**:4446–4449.
101. **Flory, J., and C. M. Radding.** 1982. Visualization of *recA* protein and its association with DNA: a priming effect of single-strand-binding protein. Cell **28**:747–756.
102. **Fogliano, M., and P. F. Schendel.** 1981. Evidence for the inducibility of the *uvrB* operon. Nature (London) **289**:196–198.
103. **Foote, R. S., S. Mitra, and B. C. Pal.** 1980. Demethylation of O^6-methylguanine in a synthetic DNA polymer by an inducible activity in *E. coli*. Biochem. Biophys. Res. Commun. **97**:654–659.
104. **Foster, P. L., and E. Eisenstadt.** 1983. Distribution and specificity of mutations induced by neocarzinostatin in the *lacI* gene of *Escherichia coli*. J. Bacteriol. **153**:379–383.
105. **Foster, P. L., E. Eisenstadt, and J. Cairns.** 1982. Random components in mutagenesis. Nature (London) **299**:365–367.
106. **Foster, P. L., E. Eisenstadt, and J. H. Miller.** 1983. Base substitution mutations induced by metabolically activated aflatoxin B_1. Proc. Natl. Acad. Sci. U.S.A. **80**:2695–2698.
107. **Fowler, R. G., L. McGinty, and K. E. Mortelmans.** 1979. Spontaneous mutational specificity of drug resistance plasmid pKM101 in *Escherichia coli*. J. Bacteriol. **140**:929–937.
108. **Franklin, W. A., K. M. Low, and W. A. Haseltine.** 1982. Alkaline lability of novel fluorescent photoproducts produced

in ultraviolet light irradiated DNA. J. Biol. Chem. **257**:13535–13543.

109. **Fuchs, R. P. P., N. Schwartz, and M. P. Duane.** 1981. Hot spots of frameshift mutations induced by the ultimate carcinogen N-acetoxy-N-2-acetylaminofluorene. Nature (London) **294**:657–659.
110. **Ganesan, A. K., P. K. Cooper, P. C. Hanawalt, and C. A. Smith.** 1982. Biochemical mechanisms and genetic control of DNA repair. Prog. Mutat. Res. **4**:313–323.
111. **Gasc, A., M. N. Sicard, J. P. Claverys, and A. M. Sicard.** 1980. Lack of SOS repair in *Streptococcus pneumoniae*. Mutat. Res. **70**:157–165.
112. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.
113. **George, J., R. Devoret, and M. Radman.** 1974. Indirect ultraviolet-reactivation of phage lambda. Proc. Natl. Acad. Sci. U.S.A. **71**:144–147.
114. **Ginsburg, H., S. H. Edmiston, J. Harper, and D. W. Mount.** 1982. Isolation and characterization of an operator-constitutive mutation in the *recA* gene of *E. coli* K-12. Mol. Gen. Genet. **187**:4–11.
115. **Glickman, B.** 1983. Mutational specificity of UV light in *E. coli*: influence of excision repair and the mutator plasmid pKM101, p. 135–170. *In* C. W. Lawrence (ed.), Induced mutagenesis. Plenum Publishing Corp., New York.
116. **Glickman, B. W., N. Guijt, and P. Morand.** 1977. The genetic characterization of *lexB32, lexB33* and *lexB35* mutations of *Escherichia coli*: location and complementation pattern for UV resistance. Mol. Gen. Genet. **157**:83–89.
117. **Gottesman, S.** 1981. Genetic control of the SOS system in *E. coli*. Cell **23**:1–2.
118. **Gottesman, S., E. Halpern, and P. Trisler.** 1981. Role of *sulA* and *sulB* in filamentation by Lon mutants of *Escherichia coli* K-12. J. Bacteriol. **148**:265–273.
119. **Goze, A., and R. Devoret.** 1979. Repair promoted by plasmid pKM101 is different from SOS repair. Mutat. Res. **61**:163–179.
120. **Greenberg, J., L. Berends, J. Donch, and B. Johnson.** 1975. Reversion studies with *exrB* in *Escherichia coli*. Genet. Res. **25**:109–117.
121. **Greener, A., and C. W. Hill.** 1980. Identification of a novel genetic element in *Escherichia coli* K-12. J. Bacteriol. **144**:312–321.
122. **Grossman, L.** 1981. Enzymes involved in the repair of damaged DNA. Arch. Biochem. Biophys. **211**:511–522.
123. **Grossman, A. D., R. R. Burgess, W. Walter, and C. A. Gross.** 1983. Mutations in the *lon* gene of *E. coli* K12 phenotypically suppress a mutation in the sigma subunit of RNA polymerase. Cell **32**:151–159.
124. **Gudas, L. J.** 1976. The induction of protein X in DNA repair and cell division mutants of *Escherichia coli*. J. Mol. Biol. **104**:567–587.
125. **Gudas, L. J., and D. W. Mount.** 1977. Identification of the *recA(tif)* gene product of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:5280–5284.
126. **Gudas, L. J., and A. B. Pardee.** 1975. Model for regulation of *Escherichia coli* DNA repair functions. Proc. Natl. Acad. Sci. U.S.A. **72**:2330–2334.
127. **Gudas, L. J., and A. B. Pardee.** 1976. DNA synthesis inhibition and the induction of protein X in *Escherichia coli*. J. Mol. Biol. **101**:459–477.
128. **Guttenplan, J. B., and S. Milstein.** 1982. Resistance of *Salmonella typhimurium* TA1535 to O^6-guanine methylation and mutagenesis induced by low doses of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine: an apparent constitutive repair activity. Carcinogenesis **3**:327–332.
129. **Hadden, C. T., R. S. Foote, and S. Mitra.** 1983. Adaptive response of *Bacillus subtilis* to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. J. Bacteriol. **153**:756–762.
130. **Hall, J. D., and D. W. Mount.** 1981. Mechanisms of DNA replication and mutagenesis in ultraviolet-irradiated bacteria and mammalian cells. Prog. Nucleic Acid Res. Mol. Biol. **25**:53–126.
131. **Hanawalt, P. C.** 1982. Perspectives on DNA repair and inducible recovery phenomena. Biochimie **64**:847–851.
132. **Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, and C. A. Smith.** 1979. DNA repair in bacteria and mammalian cells. Annu. Rev. Biochem. **48**:783–836.
133. **Hartman, P. E.** 1980. Bacterial mutagenesis: review of new insights. Environ. Mutagen. **2**:3–16.
134. **Harwood, J. H., E. Williams, and B. W. Bainbridge.** 1972. Mutation of the methane oxidizing bacterium *Methylococcus capsulatus*. J. Appl. Bacteriol. **35**:99–108.
135. **Haseltine, W. A.** 1983. Ultraviolet light repair and mutagenesis revisited. Cell **33**:13–17.
136. **Hertman, I., and S. E. Luria.** 1967. Transduction studies on the role of a rec^+ gene in ultraviolet induction of prophage lambda. J. Mol. Biol. **23**:117–133.
137. **Hickson, I. D., H. M. Arthur, D. Bramhill, and P. T. Emmerson.** 1983. The *E. coli uvrD* gene product is DNA helicase II. Mol. Gen. Genet. **190**:265–270.
138. **Hofemeister, J.** 1977. DNA repair in *Proteus mirabilis*. Mol. Gen. Genet. **154**:35–41.
139. **Hofemeister, J., and G. Eitner.** 1981. Repair and plasmid R46 mediated mutation requires inducible functions in *Proteus mirabilis*. Mol. Gen. Genet. **183**:369–375.
140. **Hofemeister, J., H. Kohler, and V. D. Filippov.** 1979. DNA repair in *Proteus mirabilis*. VI. Plasmid (R46-) mediated recovery and UV mutagenesis. Mol. Gen. Genet. **176**:265–273.
141. **Horii, T., T. Ogawa, T. Nakatani, T. Hase, H. Matsubara, and H. Ogawa.** 1981. Regulation of SOS functions: purification of *E. coli* LexA protein and determination of its specific site cleaved by the RecA protein. Cell **27**:515–522.
142. **Horii, T., T. Ogawa, and H. Ogawa.** 1981. Nucleotide sequence of the *lexA* gene of *E. coli*. Cell **23**:689–697.
143. **Howard-Flanders, P., and L. Theriot.** 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics **53**:1137–1150.
144. **Howarth, S.** 1965. Resistance to the bactericidal effect of ultraviolet radiation conferred on enterobacteria by the colicin factor Col I. J. Gen. Microbiol. **40**:43–55.
145. **Howarth, S.** 1966. Increase in frequency of ultraviolet induced mutation brought about by the colicin factor Col I in *Salmonella typhimurium*. Mutat. Res. **3**:129–134.
146. **Huisman, O., and R. d'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature (London) **290**:797–799.
147. **Ichikawa-Ryo, H., and S. Kondo.** 1975. Indirect mutagenesis in phage lambda by ultraviolet preirradiation of host bacteria. J. Mol. Biol. **97**:77–92.
148. **Inouye, M.** 1971. Pleiotrophic effect of the *recA* gene of *Escherichia coli*: uncoupling of cell division from deoxyribonucleic acid replication. J. Bacteriol. **106**:539–542.
149. **Irbe, R. M., L. M. E. Morin, and M. Oishi.** 1981. Prophage $\phi 80$ induction in *Escherichia coli* K-12 by specific deoxyoligonucleotides. Proc. Natl. Acad. Sci. U.S.A. **78**:138–142.
150. **Irbe, R. M., and M. Oishi.** 1980. Propnage induction in a permeabilized cell system: induction by deoxyribonucleases and the role of *recBC*-deoxyribonuclease. J. Bacteriol. **144**:1061–1067.
151. **Jeggo, P.** 1979. Isolation and characterization of *Escherichia coli* K-12 mutants unable to induce the adaptive response to simple alkylating agents. J. Bacteriol. **139**:783–791.
152. **Jeggo, P., M. Defais, L. Samson, and P. Schendel.** 1977. An adaptive response of *E. coli* to low levels of alkylating agent: comparison with previously characterised DNA repair pathways. Mol. Gen. Genet. **157**:1–9.
153. **Jeggo, P., M. Defais, L. Samson, and P. Schendel.** 1978. The adaptive response of *E. coli* to low levels of alkylating agent: the role of *polA* in killing adaptation. Mol. Gen. Genet. **162**:299–305.
154. **Kaina, B.** 1982. Enhanced survival and reduced mutation and aberration frequencies induced in V79 Chinese hamster cells

pre-exposed to low levels of methylating agents. Mutat. Res. **93**:195–211.
155. **Karran, P., T. Hjelmgren, and T. Lindahl.** 1982. Induction of a DNA glycosylase for N-methylated purines is part of the adaptive response to alkylating agents. Nature (London) **296**:770–773.
156. **Karran, P., T. Lindahl, and B. E. Griffin.** 1979. Adaptive response to alkylating agents involves alteration *in situ* of O^6-methylguanine residues in DNA. Nature (London) **280**:76–77.
157. **Karran, P., T. Lindahl, I. Ofsteng, G. B. Evensen, and E. Seeberg.** 1980. *Escherichia coli* mutants deficient in 3-methyladenine-DNA glycosylase. J. Mol. Biol. **140**:101–127.
158. **Karran, P., and M. G. Marinus.** 1982. Mismatch correction at O^6-methylguanine residues in *E. coli* DNA. Nature (London) **296**:868–869.
159. **Karran, P., S. Stevens, and B. Sedgwick.** 1982. The adaptive response to alkylating agents: the removal of O^6-methylguanine from DNA is not dependent on DNA polymerase-1. Mutat. Res. **104**:67–73.
160. **Karu, A. E., and E. D. Belk.** 1982. Induction of *E. coli recA* protein via *recBC* and alternate pathways: quantitation by enzyme-linked immunosorbent assay (ELISA). Mol. Gen. Genet. **185**:275–282.
161. **Kataoka, H., and M. Sekiguchi.** 1982. Are purine bases enzymatically inserted into depurinated DNA in *Escherichia coli*? J. Biochem. **92**:971–973.
162. **Kataoka, H., Y. Yamamoto, and M. Sekiguichi.** 1983. A new gene (*alkB*) of *Escherichia coli* that controls sensitivity to methyl methanesulfonate. J. Bacteriol. **153**:1301–1307.
163. **Kato, T.** 1977. Effects of chloramphenicol and caffeine on postreplication repair in *uvrA*$^-$ *umuC*$^-$ and *uvrA*$^-$ *recF*$^-$ strains of *Escherichia coli* K-12. Mol. Gen. Genet. **156**:115–120.
164. **Kato, T., T. Ise, and H. Shinagawa.** 1982. Mutational specificity of the *umuC* mediated mutagenesis in *Escherichia coli*. Biochimie **64**:731–733.
165. **Kato, T., and E. Nikano.** 1981. Effects of the *umuC36* mutation on ultraviolet-radiation-induced base change and frameshift mutagenesis in *Escherichia coli*. Mutat. Res. **83**:307–319.
166. **Kato, T., and Y. Shinoura.** 1977. Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. Mol. Gen. Genet. **156**:121–131.
167. **Kato, T., Y. Shinoura, A. Templin, and A. J. Clark.** 1980. Analysis of ultraviolet light-induced suppressor mutations in the strain of *Escherichia coli* K-12 AB1157: an implication for molecular mechanisms of UV mutagenesis. Mol. Gen. Genet. **180**:283–291.
168. **Kennedy, A., M. Fox, G. Murphy, and J. B. Little.** 1980. Relationship between X-ray exposure and malignant transformation in C3H 10T 1/2 cells. Proc. Natl. Acad. Sci. U.S.A. **77**:7262–7266.
169. **Kenyon, C. J.** 1983. The bacterial response to DNA damage. Trends Biochem. Sci. **8**:84–87.
170. **Kenyon, C. J., R. Brent, M. Ptashne, and G. C. Walker.** 1982. Regulation of damage-inducible genes in *Escherichia coli*. J. Mol. Biol. **160**:445–457.
171. **Kenyon, C. J., and G. C. Walker.** 1980. DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:2819–2823.
172. **Kenyon, C. J., and G. C. Walker.** 1981. Expression of the *E. coli uvrA* gene is inducible. Nature (London) **289**:808–810.
173. **Kimball, R. F., M. E. Boling, and S. W. Perdue.** 1977. Evidence that inducible error-prone repair is absent in *Hemophilus influenzae* Rd, with a discussion of the relation of error-prone repair of alkylating agent damage. Mutat. Res. **44**:183–196.
174. **Kogama, T., T. A. Torrey, and M. J. Connaughton.** 1979. Induction of UV-resistant DNA replication in *Escherichia coli*: induced stable DNA replication as an SOS function. Mol. Gen. Genet. **176**:1–9.
175. **Kondo, S.** 1969. Mutagenicity versus radiosensitivity in *Escherichia coli*, p. 126–127. Proceedings of the XIIth International Congress on Genetics, vol. II.
176. **Kondo, S., H. Ichikawa, K. Iwo, and T. Kato.** 1970. Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different repair capacities. Genetics **66**:187–217.
177. **Krassin, F., and F. Hutchinson.** 1981. Repair of DNA double-strand breaks in *Escherichia coli* cells requires synthesis of proteins that can be induced by UV light. Proc. Natl. Acad. Sci. U.S.A. **78**:3450–3453.
178. **Krueger, J. H., S. J. Elledge, and G. C. Walker.** 1983. Isolation and characterization of Tn*5* insertion mutations in the *lexA* gene of *Escherichia coli*. J. Bacteriol. **153**:1368–1378.
179. **Krueger, J. H., and G. C. Walker.** 1983. Mud(Ap, *lac*)-generated fusions in studies of gene expression. Methods Enzymol. **100**:501–509.
180. **Kunkel, T. A., R. M. Schaaper, and L. A. Loeb.** 1983. Depurination-induced infidelity of deoxyribonucleic acid synthesis with purified deoxyribonucleic acid replication proteins *in vitro*. Biochemistry **22**:2378–2384.
181. **Kunkel, T. A., C. W. Shearman, and L. A. Loeb.** 1981. Mutagenesis *in vitro* by depurination of ϕX174 DNA. Nature (London) **291**:349–351.
182. **Lackey, D., S. W. Krauss, and S. Linn.** 1982. Isolation of an altered form of DNA polymerase I from *Escherichia coli* cells induced for *recA*/*lexA* functions. Proc. Natl. Acad. Sci. U.S.A. **79**:330–334.
183. **Lambert, M. E., S. Gattoni-Celli, P. Kirschmeier, and I. B. Weinstein.** 1983. Benzo(*a*)pyrene induction of extrachromosomal viral DNA synthesis in rat cells transformed by polyoma virus. Carcinogenesis **4**:587–593.
184. **Langer, P. J., W. G. Shanabruch, and G. C. Walker.** 1981. Functional organization of plasmid pKM101. J. Bacteriol. **145**:1310–1316.
185. **Langer, P. J., and G. C. Walker.** 1981. Restriction endonuclease cleavage map of pKM101: relationship to parental plasmid R46. Mol. Gen. Genet. **182**:268–272.
186. **Lark, K. G., and C. A. Lark.** 1979. *recA*-dependent DNA replication in the absence of protein synthesis: characteristics of a dominant lethal replication mutation *dnaT*, and requirement for *recA*$^+$ function. Cold Spring Harbor Symp. Quant. Biol. **43**:537–549.
187. **Lavi, S.** 1982. Carcinogen-mediated activation of SV40 replicons: a model system for initiation of carcinogenesis. *In* R. T. Schimke (ed.), Gene amplification. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
188. **LeClerc, J. E., and N. L. Istock.** 1982. Specificity of UV mutagenesis in the *lac* promoter of M13*lac* hybrid phage DNA. Nature (London) **297**:596–598.
189. **Lieberman, H. B., and E. M. Witkin.** 1981. Variable expression of the *ssb-1* allele in different strains of *Escherichia coli* K-12 and B: differential suppression of its effects on DNA replication, DNA repair and ultraviolet mutagenesis. Mol. Gen. Genet. **183**:348–355.
190. **Lieberman, H. B., and E. M. Witkin.** 1983. DNA degradation, UV sensitivity and SOS-mediated mutagenesis in strains of *Escherichia coli* deficient in single-strand DNA binding protein: effects of mutations and treatments that alter levels of exonuclease V or *recA* protein. Mol. Gen. Genet. **190**:92–100.
191. **Lindahl, T.** 1976. New class of enzymes acting on damaged DNA. Nature (London) **259**:64–66.
192. **Lindahl, T.** 1982. DNA repair enzymes. Annu. Rev. Biochem. **51**:61–87.
193. **Lindahl, T., B. Demple, and P. Robins.** 1982. Suicide inactivation of the *E. coli* O^6-methylguanine-DNA methyltransferase. EMBO (Eur. Mol. Biol. Org.) J. **1**:1359–1363.
194. **Lindahl, T., B. Rydberg, T. Hjelmgren, M. Olsson, and A. Jacobsson.** 1982. Cellular defense mechanisms against alkylation of DNA, p. 89–102. *In* J. F. Lemontt and W. M. Generoso (ed.), Molecular and cellular mechanisms of mutagenesis. Plenum Publishing Corp., New York.
195. **Lippke, J. A., L. K. Gordon, D. E. Brash, and W. A. Haseltine.** 1981. Distribution of UV light-induced damage in a defined sequence of human DNA: detection of alkaline-sensitive lesions at pyrimidine nucleoside-cytidine sequences. Proc.

Natl. Acad. Sci. U.S.A. **78**:3388–3392.
196. **Little, J. W.** 1982. Control of the SOS regulatory system by the level of *recA* protease. Biochimie **64**:585–589.
197. **Little, J. W.** 1983. The SOS regulatory system: control of its state by the level of *recA* protease. J. Mol. Biol. **167**:791–808.
198. **Little, J. W., S. H. Edmiston, L. Z. Pacelli, and D. W. Mount.** 1980. Cleavage of the *Escherichia coli lexA* protein by the *recA* protease. Proc. Natl. Acad. Sci. U.S.A. **77**:3225–3229.
199. **Little, J. W., and P. C. Hanawalt.** 1977. Induction of protein X in *Escherichia coli*. Mol. Gen. Genet. **150**:237–248.
200. **Little, J. W., and J. E. Harper.** 1979. Identification of the *lexA* gene product of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:6147–6151.
201. **Little, J. W., and D. G. Kleid.** 1977. *Escherichia coli* protein X is the *recA* gene product. J. Biol. Chem. **252**:6251–6252.
202. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
203. **Little, J. W., D. W. Mount, and C. R. Yanisch-Perron.** 1981. Purified *lexA* protein is a repressor of the *recA* and *lexA* genes. Proc. Natl. Acad. Sci. U.S.A. **78**:4199–4203.
204. **Livneh, Z.** 1983. Directed mutagenesis method for analysis of mutagen specificity: application to ultraviolet-induced mutagenesis. Proc. Natl. Acad. Sci. U.S.A. **80**:237–241.
205. **Livneh, Z., D. Elad, and J. Sperling.** 1979. Enzymatic insertion of purine bases into depurinated DNA *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **76**:1089–1093.
206. **Lloyd, R. G.** 1983. *lexA* dependent recombination in *uvrD* strains of *Escherichia coli*. Mol. Gen. Genet. **189**:157–161.
207. **Lloyd, R. G., S. M. Picksley, and C. Prescott.** 1983. Inducible expression of a gene specific to the *recF* pathway for recombination in *Escherichia coli* K12. Mol. Gen. Genet. **190**:162–167.
208. **Loeb, L. A., and T. A. Kunkel.** 1982. Fidelity of DNA synthesis. Annu. Rev. Biochem. **52**:429–457.
209. **Loveless, A.** 1969. Possible relevance of O^6-alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides. Nature (London) **223**:206–207.
210. **Lovett, S. T., and A. J. Clark.** 1983. Genetic analysis of regulation of the *recF* pathway of recombination in *Escherichia coli* K-12. J. Bacteriol. **153**:1471–1478.
211. **Lu, A.-L., S. Clark, and P. Modrich.** 1983. Methyl-directed repair of DNA base-pair mismatches *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **80**:4639–4643.
212. **MacPhee, D. G.** 1973. Effect of *rec* mutations on the ultraviolet protecting and mutation-enhancing properties of the plasmid R-Utrecht in *Salmonella typhimurium*. Mutat. Res. **19**:356–359.
213. **Maenhaut-Michel, G., and P. Caillet-Fauquet.** 1982. 2-Aminopurine induced DNA repair in *E. coli*. Mol. Gen. Genet. **188**:143–148.
214. **Marinus, M. G., and N. R. Morris.** 1973. Isolation of deoxyribonucleic acid methylase mutants of *Escherichia coli* K-12. J. Bacteriol. **114**:1143–1150.
215. **Markham, B. E., J. W. Little, and D. W. Mount.** 1981. Nucleotide sequence of the *lexA* gene of *Escherichia coli* K-12. Nucleic Acids Res. **9**:4149–4161.
216. **McCann, J., and B. N. Ames.** 1976. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals: discussion. Proc. Natl. Acad. Sci. U.S.A. **73**:950–954.
217. **McCann, J., E. Choi, E. Yamasaki, and B. N. Ames.** 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. U.S.A. **72**:5135–5139.
218. **McCann, J., N. E. Spingarn, J. Kobori, and B. N. Ames.** 1975. Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids. Proc. Natl. Acad. Sci. U.S.A. **72**:979–983.
219. **McEntee, K.** 1977. Protein X is the product of the *recA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:5275–5279.
220. **McEntee, K., and G. M. Weinstock.** 1981. *tif-1* mutation alters polynucleotide recognition by the *recA* protein of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:6061–6065.
221. **McEntee, K., G. M. Weinstock, and I. R. Lehman.** 1979. Initiation of general recombination catalyzed *in vitro* by the RecA protein of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:2615–2619.
222. **McPartland, A., L. Green, and H. Echols.** 1980. Control of *recA* gene RNA in *E. coli*: regulatory and signal genes. Cell **20**:731–737.
223. **Mehta, J. A., D. B. Ludlum, A. Renard, and W. G. Verly.** 1981. Repair of O^6-ethylguanine in DNA by a chromatin fraction from rat liver: transfer of the ethyl group to an acceptor protein. Proc. Natl. Acad. Sci. U.S.A. **78**:6766–6770.
224. **Meyer, R. R., J. Glassberg, and A. Kornberg.** 1979. An *Escherichia coli* mutant defective in single-stranded binding protein is defective in DNA replication. Proc. Natl. Acad. Sci. U.S.A. **76**:1702–1705.
225. **Meyer, R. R., D. C. Rein, and J. Glassberg.** 1982. The product of the *lexC* gene of *Escherichia coli* is single-stranded DNA-binding protein. J. Bacteriol. **150**:433–435.
226. **Meyer, R. R., D. W. Voegele, S. M. Ruben, D. C. Rein, and J. M. Trela.** 1982. Influence of single-stranded DNA-binding protein on *recA* induction in *Escherichia coli*. Mutat. Res. **94**:299–313.
227. **Miki, T., Y. Ebina, F. Kishi, and A. Nakazawa.** 1981. Organization of the *lexA* gene of *Escherichia coli* and nucleotide sequence of the regulatory region. Nucleic Acids Res. **9**:529–543.
228. **Miller, J. H.** 1982. Carcinogens induce targeted mutations in *Escherichia coli*. Cell **31**:5–7.
229. **Miller, H. I., M. Kirk, and H. Echols.** 1981. SOS induction and autoregulation of the *himA* gene for site-specific recombination in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:6754–6758.
230. **Miller, H. I., and H. A. Nash.** 1981. Direct role of the *himA* gene product in phage integration. Nature (London) **290**:523–526.
231. **Miller, J. H., C. Coulondre, and P. J. Farabaugh.** 1978. Correlation of nonsense sites in the *lacI* gene with specific codons in the nucleotide sequence. Nature (London) **274**:770–775.
232. **Mitra, S., B. C. Pal, and R. S. Foote.** 1982. O^6-Methylguanine-DNA methyltransferase in wild-type and *ada* mutants of *Escherichia coli*. J. Bacteriol. **152**:534–537.
233. **Miura, A., and J. Tomizawa.** 1968. Studies on radiation-sensitive mutants of *E. coli*. III. Participation of the Rec system in induction of mutation by ultraviolet irradiation. Mol. Gen. Genet. **103**:1–10.
234. **Mizusawa, S., and S. Gottesman.** 1983. Protein degradation in *Escherichia coli*: the *lon* gene controls the stability of *sulA* protein. Proc. Natl. Acad. Sci. U.S.A. **80**:358–362.
235. **Mizusawa, H., C.-H. Lee, T. Kafefuda, K. McKenney, H. Shimatake, and M. Rosenberg.** 1981. Base insertion and deletion mutations induced in an *Escherichia coli* plasmid by benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide. Proc. Natl. Acad. Sci. U.S.A. **78**:6817–6820.
236. **Molina, A. M., N. Babudri, M. Tamaro, S. Venturini, and C. Monti-Bragadin.** 1979. *Enterobacteriaceae* plasmids enhancing chemical mutagenesis and their distribution among incompatibility groups. FEMS Microbiol. Lett. **5**:33–37.
237. **Monti-Bragadin, C., N. Babudri, and L. Samer.** 1976. Expression of the plasmid pKM101-determined repair system in $recA^-$ and lex^- strains of *Escherichia coli*. Mol. Gen. Genet. **145**:303–306.
238. **Moore, P. D., K. K. Bose, S. D. Rabkin, and B. S. Strauss.** 1981. Sites of termination of *in vitro* DNA synthesis on ultraviolet- and *N*-acetylaminofluorene-treated ϕX174 templates by prokaryotic and eukaryotic DNA polymerases. Proc. Natl. Acad. Sci. U.S.A. **78**:110–114.
239. **Moore, P. D., S. D. Rabkin, A. L. Osborn, C. M. King, and B. S. Strauss.** 1982. Effect of acetylated and deacetylated 2-aminofluorene adducts on *in vitro* DNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **79**:7166–7170.
240. **Moore, P. D., S. D. Rabkin, A. L. Osborn, and B. S. Strauss.** 1982. Interactions between DNA polymerase and aminofluorene adducts that affect the recognition and possibly the mutagenicity of the lesions. Biochimie **64**:757–762.
241. **Moore, P. D., S. D. Rabkin, and B. S. Strauss.** 1982. *In vitro*

replication of mutagen-damaged DNA: sites of termination, p. 179–197. *In* J. F. Lemontt and W. M. Generoso (ed.), Molecular and cellular mechanisms of mutagenesis. Plenum Publishing Corp., New York.

242. **Morand, P., M. Blanco, and R. Devoret.** 1977. Characterization of *lexB* mutations in *Escherichia coli* K-12. J. Bacteriol. **131**:572–582.
243. **Moreau, P. L., M. Fanica, and R. Devoret.** 1980. Induction of prophage lambda does not require full induction of *recA* protein synthesis. Biochimie **62**:687–694.
244. **Moreau, P. L., J. V. Pelico, and R. Devoret.** 1982. Cleavage of lambda repressor and synthesis of *recA* protein induced by transferred UV-damaged F sex factor. Mol. Gen. Genet. **186**:170–179.
245. **Morohoshi, F., and N. Munakata.** 1983. Adaptive response to simple alkylating agents in *Bacillus subtilis* cells. Mutat. Res. **110**:23–37.
246. **Mortelmans, K. E., and B. A. D. Stocker.** 1976. Ultraviolet light protection, enhancement of ultraviolet light mutagenesis, and mutator effect of plasmid R46 in *Salmonella typhimurium*. J. Bacteriol. **128**:271–282.
247. **Mortelmans, K. E., and B. A. D. Stocker.** 1979. Segregation of the mutator property of plasmid R46 from its ultraviolet-protecting property. Mol. Gen. Genet. **167**:317–328.
248. **Mount, D. W.** 1971. Isolation and genetic analysis of a strain of *Escherichia coli* K-12 with an amber *recA* mutation. J. Bacteriol. **107**:388–389.
249. **Mount, D. W.** 1977. A mutant of *Escherichia coli* showing constitutive expression of the lysogenic induction and error-prone DNA repair pathways. Proc. Natl. Acad. Sci. U.S.A. **74**:300–304.
250. **Mount, D. W.** 1979. Isolation and characterization of mutants of λ*recA* which synthesize a hyperactive *recA* protein. Virology **98**:484–488.
251. **Mount, D. W., C. Kosel, and A. Walker.** 1976. Inducible error-free DNA repair in *tsl recA* mutants of *E. coli*. Mol. Gen. Genet. **146**:37–42.
252. **Mount, D. W., K. B. Low, and S. Edmiston.** 1972. Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. **112**:886–893.
253. **Mount, D. W., A. C. Walker, and C. Kosel.** 1973. Suppression of *lex* mutations affecting deoxyribonucleic acid repair in *Escherichia coli* K-12 by closely linked thermosensitive mutations. J. Bacteriol. **116**:950–956.
254. **Nasland, M., D. Segerback, and A. Kolman.** 1983. *S*-Adenosylmethionine, an endogenous alkylating agent. Mutat. Res. **119**:229–232.
255. **Neidhardt, F. C., and R. A. Van Bogelen.** 1981. Positive regulatory gene for temperature-controlled proteins in *Escherichia coli*. Biochem. Biophys. Res. Commun. **100**:894–900.
256. **Neidhardt, F. C., R. A. Van Bogelen, and E. T. Lau.** 1983. Molecular cloning and expression of a gene that controls the high-temperature regulon of *Escherichia coli*. J. Bacteriol. **153**:597–603.
257. **Notani, N. K., and J. E. Setlow.** 1980. Inducible repair system in *Haemophilus influenzae*. J. Bacteriol. **143**:516–519.
258. **Oeda, K., T. Horiuchi, and M. Sekiguchi.** 1982. The *uvrD* gene of *E. coli* encodes a DNA-dependent ATPase. Nature (London) **298**:98–100.
259. **Olsson, M., and T. Lindahl.** 1980. Repair of alkylated DNA in *Escherichia coli*: methyl group transfer from O^6-methylguanine to a protein cysteine residue. J. Biol. Chem. **255**:10569–10571.
260. **Osborn, M., S. Person, S. Phillips, and F. Funk.** 1967. A determination of mutagen specificity in bacteria using nonsense mutants of bacteriophage T4. J. Mol. Biol. **26**:437–447.
261. **Overbye, K. M., and P. Margolin.** 1981. The role of the *supX* gene in ultraviolet-light-induced mutagenesis in *Salmonella typhimurium*. J. Bacteriol. **146**:170–178.
262. **Pacelli, L. Z., S. H. Edmiston, and D. W. Mount.** 1979. Isolation and characterization of amber mutations in the *lexA* gene of *Escherichia coli* K-12. J. Bacteriol. **137**:568–573.
263. **Pang, P. P., and G. C. Walker.** 1983. The *Salmonella typhimurium* LT2 *uvrD* gene is regulated by the *lexA* gene product. J. Bacteriol. **154**:1502–1504.
264. **Pegg, A. E., M. Roberfroid, C. von Bahr, R. S. Foote, S. Mitra, H. Bresil, A. Likhachev, and R. Montesano.** 1982. Removal of O^6-methylguanine from DNA by human liver fractions. Proc. Natl. Acad. Sci. U.S.A. **79**:5162–5165.
265. **Perry, K. L., and G. C. Walker.** 1982. Identification of plasmid(pKM101)-coded proteins involved in mutagenesis and UV resistance. Nature (London) **300**:278–281.
266. **Philippe, M., T. S. F. Wang, P. C. Hanawalt, and D. Korn.** 1982. DNA synthesis on UV irradiated model templates using human DNA polymerases α and β: primer slippage to account for evident transdimer continuity in product. Biochimie **64**:783–788.
267. **Phizicky, E. M., and J. W. Roberts.** 1980. Kinetics of RecA protein-directed inactivation of repressors of phage lambda and phage P22. J. Mol. Biol. **139**:319–328.
268. **Phizicky, E. M., and J. W. Roberts.** 1981. Induction of SOS functions: regulation of proteolytic activity of *E. coli* Rec protein by interaction with DNA and nucleoside triphosphate. Cell **25**:259–267.
269. **Pollard, E. C., D. J. Fluke, and D. Kazanis.** 1981. Induced radioresistance: an aspect of induced repair. Mol. Gen. Genet. **184**:421–429.
270. **Pollard, E., and E. P. Randall.** 1973. Studies on the inducible inhibitor of radiation-induced DNA degradation of *E. coli*. Radiat. Res. **55**:265–279.
271. **Pukkila, P. J., J. Peterson, G. Herman, P. Modrich, and M. Meselson.** 1983. Effects of high levels of DNA adenine methylation on methyl-directed mismatch repair in *Escherichia coli*. Genetics **104**:571–582.
272. **Quillardet, P., and R. Devoret.** 1982. Damaged-site independent mutagenesis of phage lambda produced by inducible error-prone repair. Biochimie **64**:789–796.
273. **Quillardet, P., O. Huisman, R. D'Ari, and M. Hofnung.** 1982. SOS chromotest, a direct assay of induction of an SOS function in *Escherichia coli* K-12 to measure genotoxicity. Proc. Natl. Acad. Sci. U.S.A. **79**:5971–5975.
274. **Quillardet, P., P. L. Moreau, H. Ginsburg, D. W. Mount, and R. Devoret.** 1982. Cell survival, UV-reactivation and induction of prophage lambda in *Escherichia coli* K12 overproducing *recA* protein. Mol. Gen. Genet. **188**:37–43.
275. **Rabkin, S. D., P. D. Moore, and B. S. Strauss.** 1983. *In vitro* bypass of UV-induced lesions by *Escherichia coli* DNA polymerase I: specificity of nucleotide incorporation. Proc. Natl. Acad. Sci. U.S.A. **80**:1541–1545.
276. **Radman, M.** 1974. Phenomenology of an inducible mutagenic DNA repair pathway in *Escherichia coli*: SOS repair hypothesis, p. 128–142. *In* L. Prakash, F. Sherman, M. Miller, C. Lawrence, and H. W. Tabor (ed.), Molecular and environmental aspects of mutagenesis. Charles C Thomas, Publisher, Springfield, Ill.
277. **Radman, M.** 1975. SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis, p. 355–367. *In* P. Hanawalt and R. B. Setlow (ed.), Molecular mechanisms for repair of DNA, part A. Plenum Publishing Corp., New York.
278. **Radman, M., R. E. Wagner, Jr., B. W. Glickman, and M. Meselson.** 1980. DNA methylation mismatch correction and genetic stability, p. 121–130. *In* M. Alecevic (ed.), Progress in environmental mutagenesis. Elsevier/North-Holland Biomedical Press, Amsterdam.
279. **Resnick, J., and R. Sussman.** 1982. *Escherichia coli* single-strand DNA binding protein from wild type and *lexC113* mutant affects *in vitro* proteolytic cleavage of phage lambda repressor. Proc. Natl. Acad. Sci. U.S.A. **79**:2832–2835.
280. **Riazzudin, S., and T. Lindahl.** 1978. Properties of 3-methyladenine-DNA glycosylase from *E. coli*. Biochemistry **17**:2110–2118.
281. **Roberts, J. W., and R. Devoret.** 1983. Lysogenic induction, p. 123–144. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

282. **Roberts, J. W., E. M. Phizicky, D. G. Burbee, C. W. Roberts, and P. L. Moreau.** 1982. A brief consideration of the SOS inducing signal. Biochimie **64**:805–807.
283. **Roberts, J. W., and C. W. Roberts.** 1975. Proteolytic cleavage of bacteriophage lambda repressor in induction. Proc. Natl. Acad. Sci. U.S.A. **72**:147–151.
284. **Roberts, J. W., and C. W. Roberts.** 1981. Two mutations that alter the regulatory activity of *E. coli recA* protein. Nature (London) **290**:422–424.
285. **Roberts, J. W., C. W. Roberts, and N. L. Craig.** 1978. *Escherichia coli recA* gene product inactivates phage lambda repressor. Proc. Natl. Acad. Sci. U.S.A. **75**:4714–4718.
286. **Roberts, J. W., C. W. Roberts, and D. W. Mount.** 1977. Inactivation and proteolytic cleavage of phage lambda repressor *in vitro* in an ATP-dependent reaction. Proc. Natl. Acad. Sci. U.S.A. **74**:2283–2287.
287. **Robins, P., and J. Cairns.** 1979. The numerology of the adaptive response to alkylating agents. Nature (London) **280**:74–76.
288. **Rosner, J. L., L. R. Kass, and M. B. Yarmolinsky.** 1968. Parallel behavior of F and Pl in causing indirect induction of lysogenic bacteria. Cold Spring Harbor Symp. Quant. Biol. **33**:785–789.
289. **Rupp, W. D., and P. Howard-Flanders.** 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31**:291–304.
290. **Rydberg, B., and T. Lindahl.** 1982. Nonenzymatic methylation of DNA by the intracellular methyl group donor *S*-adenosyl-L-methionine is a potentially mutagenic reaction. EMBO (Eur. Mol. Biol. Org.) J. **1**:211–216.
291. **Salles, B., and C. Paoletti.** 1983. Control of UV induction of *recA* protein. Proc. Natl. Acad. Sci. U.S.A. **80**:65–69.
292. **Salles, B., C. Paoletti, and G. Villani.** 1983. Lack of single-strand DNA-binding protein amplification under conditions of SOS induction in *E. coli*. Mol. Gen. Genet. **189**:175–177.
293. **Samson, L., and J. Cairns.** 1977. A new pathway for DNA repair in *Escherichia coli*. Nature (London) **267**:281–282.
294. **Samson, L., and J. L. Schwartz.** 1980. Evidence for an adaptive DNA repair pathway in CHO and human skin fibroblast cell lines. Nature (London) **287**:861–863.
295. **Sancar, A., and W. D. Rupp.** 1983. A novel repair enzyme: UVRABC excision nuclease of *Escherichia coli* cuts a DNA strand on both sides of the damaged region. Cell **33**:249–260.
296. **Sancar, G. B., A. Sancar, J. W. Little, and W. D. Rupp.** 1982. The *uvrB* gene of *Escherichia coli* has both *lexA*-repressed and *lexA*-independent promoters. Cell **28**:523–530.
297. **Sancar, A., G. B. Sancar, and W. D. Rupp.** 1982. *LexA* protein inhibits transcription of the *E. coli uvrA* gene *in vitro*. Nature (London) **298**:96–98.
298. **Sarasin, A., F. Bourre, and A. Benoit.** 1982. Error-prone replication of ultraviolet-irradiated simian virus 40 in carcinogen-treated monkey kidney cells. Biochimie **64**:815–821.
299. **Sargentini, N. J., R. C. Bockrath, and K. C. Smith.** 1982. Three mechanisms for ultraviolet radiation mutagenesis in *Escherichia coli* K-12 *uvrB5*: specificity for the production of back and suppressor mutants. Mutat. Res. **106**:217–224.
300. **Sargentini, N. J., and K. C. Smith.** 1981. Much of spontaneous mutagenesis in *Escherichia coli* is due to error-prone DNA repair: implications for spontaneous carcinogenesis. Carcinogenesis **2**:863–872.
301. **Sauer, R. T., R. R. Yocum, R. F. Doolittle, M. Lewis, and C. O. Pabo.** 1982. Homology among DNA-binding proteins suggests use of a conserved super-secondary structure. Nature (London) **298**:447–451.
302. **Schaaper, R. M., and B. W. Glickman.** 1982. Mutability of bacteriophage M13 by ultraviolet light: role of pyrimidine dimers. Mol. Gen. Genet. **185**:404–407.
303. **Schaaper, R. M., B. W. Glickman, and L. A. Loeb.** 1982. Role of depurination in mutagenesis by chemical carcinogens. Cancer Res. **42**:3480–3485.
304. **Schaaper, R. M., B. W. Glickman, and L. A. Loeb.** 1982. Mutagenesis resulting from depurination is an SOS process. Mutat. Res. **106**:1–9.
305. **Schaaper, R. M., T. A. Kunkel, and L. A. Loeb.** 1983. Infidelity of DNA synthesis associated with bypass of apurinic sites. Proc. Natl. Acad. Sci. U.S.A. **80**:487–491.
306. **Schaaper, R. M., and L. A. Loeb.** 1981. Depurination causes mutations in SOS-induced cells. Proc. Natl. Acad. Sci. U.S.A. **78**:1773–1777.
307. **Schendel, P. F.** 1981. Inducible repair systems and their implications for toxicology. Crit. Rev. Toxicol. **9**:311–362.
308. **Schendel, P. F., and M. Defais.** 1980. The role of *umuC* gene product in mutagenesis by simple alkylating agents. Mol. Gen. Genet. **177**:661–665.
309. **Schendel, P. F., M. Defais, P. Jeggo, L. Samson, and J. Cairns.** 1978. Pathways of mutagenesis and repair in *Escherichia coli* exposed to low levels of simple alkylating agents. J. Bacteriol. **135**:466–475.
310. **Schendel, P. F., and P. E. Robins.** 1978. Repair of O^6-methylguanine in adapted *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **75**:6017–6020.
311. **Schumann, J. P., D. T. Jones, and D. R. Woods.** 1982. UV light induction of proteins in *Bacteroides fragilis* under anaerobic conditions. J. Bacteriol. **151**:44–47.
312. **Schuster, H., D. Beyersmann, M. Mikolajczyk, and M. Schlicht.** 1973. Prophage induction by high temperature in thermosensitive *dna* mutants lysogenic for bacteriophage lambda. J. Virol. **11**:879–885.
313. **Schwartz, J. L., and L. Samson.** 1983. Mutation induction in Chinese hamster ovary cells after chronic pretreatment with MNNG. Mutat. Res. **119**:393–397.
314. **Sedgwick, B.** 1982. Genetic mapping of *ada* and *adc* mutations affecting the adaptive response of *Escherichia coli* to alkylating agents. J. Bacteriol. **150**:984–988.
315. **Sedgwick, B.** 1983. Molecular cloning of a gene which regulates the adaptive response to alkylating agents in *Escherichia coli*. Mol. Gen. Genet. **191**:466–472.
316. **Sedgwick, B., and T. Lindahl.** 1982. A common mechanism for repair of O^6-methylguanine and O^6-ethylguanine in DNA. J. Mol. Biol. **154**:169–175.
317. **Sedgwick, B., and P. Robins.** 1980. Isolation of mutants of *Escherichia coli* with increased resistance to alkylating agents: mutants deficient in thiols and mutants constitutive for the adaptive response. Mol. Gen. Genet. **180**:85–90.
318. **Sedgwick, S. G., G. T. Yarranton, and R. W. Heath.** 1981. Lysogenic induction of lambdoid phages in *lexA* mutants of *Escherichia coli*. Mol. Gen. Genet. **184**:457–459.
319. **Sedliakova, M., V. Slezarikova, J. Brozmanova, F. Masek, and V. Bayerova.** 1980. Role of UV-inducible proteins in repair of various wild-type *Escherichia coli* cells. Mutat. Res. **71**:15–23.
320. **Sedliakova, M., V. Slezarikova, and M. Pirsel.** 1978. UV-inducible repair. II. Its role in various defective mutants of *Escherichia coli* K-12. Mol. Gen. Genet. **167**:209–215.
321. **Seeberg, E., and A.-L. Steinum.** 1982. Purification and properties of the *uvrA* protein from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **79**:988–992.
322. **Seeberg, E., A.-L. Steinum, and O. R. Blingsmo.** 1982. Two separable protein species which both restore *uvrABC* endonuclease activity in extracts from *uvrC* mutated cells. Biochimie **64**:825–828.
323. **Shanabruch, W. G., R. P. Rein, I. Behlau, and G. C. Walker.** 1983. Mutagenesis by methylating and ethylating agents in *mutH, mutL, mutS*, and *uvrD* mutants of *Salmonella typhimurium* LT2. J. Bacteriol. **153**:33–44.
324. **Shanabruch, W. G., and G. C. Walker.** 1980. Localization of the plasmid (pKM101) gene(s) involved in $recA^+lexA^+$-dependent mutagenesis. Mol. Gen. Genet. **179**:289–297.
325. **Shibata, T., C. DasGupta, R. P. Cunningham, and C. M. Radding.** 1979. Purified *Escherichia coli* RecA protein catalyzes homologous pairing of superhelical DNA and single-stranded fragments. Proc. Natl. Acad. Sci. U.S.A. **76**:1638–1642.
326. **Shinagawa, H., T. Kato, T. Ise, K. Makino, and A. Nakata.**

1983. Cloning and characterization of the *umu* operon responsible for inducible mutagenesis in *Escherichia coli*. Gene **23**:167–174.

327. **Shurvinton, C. E., and R. G. Lloyd.** 1982. Damage to DNA induces expression of the *ruv* gene of *Escherichia coli*. Mol. Gen. Genet. **185**:352–355.
328. **Siegel, E. C.** 1983. The *Escherichia coli uvrD* gene is inducible by DNA damage. Mol. Gen. Genet. **191**:397–400.
329. **Skavronskaya, A. G., N. F. Stepanova, and I. V. Andreeva.** 1982. UV-mutable hybrids of *Salmonella* incorporating *Escherichia coli* region adjacent to tryptophan operon. Mol. Gen. Genet. **185**:315–318.
330. **Sklar, R.** 1978. Enhancement of nitrosoguanidine mutagenesis by chloramphenicol in *Escherichia coli* K-12. J. Bacteriol. **136**:460–462.
331. **Sklar, R., and B. Strauss.** 1980. Role of the *uvrE* gene product and of inducible O^6-methylguanine removal in the induction of mutations by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in *Escherichia coli*. J. Mol. Biol. **143**:343–362.
332. **Sklar, R., and B. Strauss.** 1981. Removal of O^6-methylguanine from DNA of normal and xeroderma pigmentosum-derived lymphoblastoid lines. Nature (London) **289**:417–420.
333. **Steinborn, G.** 1978. Uvm mutants of *Escherichia coli* K12 deficient in UV mutagenesis. I. Isolation of *uvm* mutants and their phenotypical characterization in DNA repair and mutagenesis. Mol. Gen. Genet. **165**:87–93.
334. **Steinborn, G.** 1979. Uvm mutants of *Escherichia coli* K12 deficient in UV mutagenesis. II. Further evidence for a novel function in error-prone repair. Mol. Gen. Genet. **175**:203–208.
335. **Strauss, B., S. Rabkin, D. Sagher, and P. Moore.** 1982. The role of DNA polymerase in base substitution mutagenesis on non-instructional templates. Biochimie **64**:829–838.
336. **Streisinger, G., Y. Okada, J. Emrich, J. Newton, A. Tsugita, E. Terzaghi, and M. Inouye.** 1966. Frameshift mutations and the genetic code. Cold Spring Harbor Symp. Quant. Biol. **31**:77–84.
337. **Sutherland, B. M.** 1981. Photoreactivating enzymes. The enzymes **14**:481–515.
338. **Sweet, D. M., and B. E. B. Mosley.** 1974. Accurate repair of ultraviolet-induced damage in *Micrococcus radiodurans*. Mutat. Res. **23**:311–318.
339. **Swenberg, J. A., M. A. Bedell, K. C. Billings, D. R. Umbenhauer, and A. E. Pegg.** 1982. Cell-specific differences in O^6-alkylguanine DNA repair activity during continuous exposure to carcinogen. Proc. Natl. Acad. Sci. U.S.A. **79**:5499–5502.
340. **Swenson, P. A., and R. L. Schenley.** 1974. Respiration, growth and viability of repair-deficient mutants of *Escherichia coli* after ultraviolet irradiation. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. **25**:51–60.
341. **Tempest, P. R., and B. E. B. Mosley.** 1982. Lack of ultraviolet mutagenesis in radiation-resistant bacteria. Mutat. Res. **104**:275–280.
342. **Tessman, E., and P. K. Peterson.** 1980. *tif*-dependent induction of colicin E1, prophage lambda, and filamentation in *Escherichia coli* K-12. J. Bacteriol. **143**:1307–1317.
343. **Thoms, B., and W. Wackernagel.** 1982. UV-induced alleviation of lambda restriction in *Escherichia coli* K-12: kinetics of induction and specificity of this SOS function. Mol. Gen. Genet. **186**:111–117.
344. **Todd, P. A., and B. W. Glickman.** 1982. Mutational specificity of UV light in *Escherichia coli*: indications for a role of DNA secondary structure. Proc. Natl. Acad. Sci. U.S.A. **79**:4123–4127.
345. **Uhlin, B. E., and A. J. Clark.** 1981. Overproduction of the *Escherichia coli recA* protein without stimulation of its proteolytic activity. J. Bacteriol. **148**:386–390.
346. **Uhlin, B. E., M. R. Volkert, A. J. Clark, A. Sancar, and W. D. Rupp.** 1982. Nucleotide sequence of a *recA* operator mutation. Mol. Gen. Genet. **185**:251–254.
347. **Upton, C., and R. J. Pinney.** 1983. Expression of eight unrelated Muc^+ plasmids in eleven DNA repair-deficient *E. coli* strains. Mutat. Res. **112**:261–273.
348. **Vales, L. D., J. W. Chase, and J. B. Murphy.** 1980. Effect of *ssbA1* and *lexC113* mutations on lambda prophage induction, bacteriophage growth, and cell survival. J. Bacteriol. **143**:887–896.
349. **van den Elzen, P. J. M., J. Maat, H. H. B. Walters, E. Veltkamp, and H. J. J. Nijkamp.** 1982. The nucleotide sequence of the bacteriocin promoters of plasmids Clo DF13 and ColE1: role of *lexA* repressor and cAMP in the regulation of promoter activity. Nucleic Acids Res. **10**:1913–1928.
350. **Volkert, M. R., L. J. Margossian, and A. J. Clark.** 1981. Evidence that *rnmB* is the operator of the *Escherichia coli recA* gene. Proc. Natl. Acad. Sci. U.S.A. **78**:1786–1790.
351. **von Wright, A., and B. A. Bridges.** 1981. Effect of *gyrB*-mediated changes in chromosome structure on killing of *Escherichia coli* by ultraviolet light: experiments with strains differing in deoxyribonucleic acid repair capacity. J. Bacteriol. **146**:18–23.
352. **Waldstein, E. A., E.-H. Cao, and R. B. Setlow.** 1982. Adaptive resynthesis of O^6-methylguanine-accepting protein can explain the differences between mammalian cells proficient and deficient in methyl excision repair. Proc. Natl. Acad. Sci. U.S.A. **79**:5117–5121.
353. **Waleh, N. S., and B. A. D. Stocker.** 1979. Effect of host *lex*, *recA*, *recF*, and *uvrD* genotypes on the ultraviolet light-protecting and related properties of plasmid R46 in *Escherichia coli*. J. Bacteriol. **137**:830–838.
354. **Walker, G. C.** 1977. Plasmid (pKM101)-mediated enhancement of repair and mutagenesis: dependence on chromosomal genes in *Escherichia coli* K-12. Mol. Gen. Genet. **152**:93–103.
355. **Walker, G. C.** 1978. Isolation and characterization of mutants of the plasmid pKM101 deficient in their ability to enhance mutagenesis and repair. J. Bacteriol. **133**:1203–1211.
356. **Walker, G. C.** 1978. Inducible reactivation and mutagenesis of UV-irradiated bacteriophage P22 in *Salmonella typhimurium* LT2 containing the plasmid pKM101. J. Bacteriol. **135**:415–421.
357. **Walker, G. C.** 1979. Mutagenesis- and repair-enhancing activities associated with the plasmid pKM101. Cold Spring Harbor Symp. Quant. Biol. **43**:893–896.
358. **Walker, G. C.** 1983. Molecular principles underlying the Ames *Salmonella*-microsome test: elements and design of short term test, p. 15–39. *In* A. R. Kolber, T. K. Wang, L. D. Grant, R. S. DeWoskink, and T. J. Hughes (ed.), *In vitro* toxicology testing of environmental agents: current and future possibilities. Plenum Publishing Corp., New York.
359. **Walker, G. C.** 1983. Regulation and function of cellular gene products involved in UV and chemical mutagenesis in *E. coli*, p. 181–198. *In* C. W. Lawrence (ed.), Induced mutagenesis: molecular mechanisms and their implications for environmental protection. Plenum Publishing Corp., New York.
360. **Walker, G. C., and P. P. Dobson.** 1979. Mutagenesis and repair deficiencies of *Escherichia coli umuC* mutants are suppressed by the plasmid pKM101. Mol. Gen. Genet. **172**:17–24.
361. **Walker, G. C., S. J. Elledge, C. J. Kenyon, J. H. Krueger, and K. L. Perry.** 1982. Mutagenesis and other responses induced by DNA damage in *Escherichia coli*. Biochimie **64**:607–610.
362. **Walker, G. C., C. J. Kenyon, A. Bagg, S. J. Elledge, K. L. Perry, and W. G. Shanabruch.** 1982. Regulation and functions of *Escherichia coli* genes induced by DNA damage, p. 43–63. *In* J. F. Lemontt and W. M. Generoso (ed.), Molecular and cellular mechanisms of mutagenesis. Plenum Publishing Corp., New York.
363. **Weigle, J. J.** 1953. Induction of mutation in a bacterial virus. Proc. Natl. Acad. Sci. U.S.A. **39**:628–636.
364. **Weinstock, G. M., and K. McEntee.** 1981. *RecA* protein dependent proteolysis of bacteriophage lambda repressor. Characterization of the reaction and stimulation by DNA-binding proteins. J. Biol. Chem. **256**:10883–10888.
365. **West, S. C., E. Cassuto, J. Mursalim, and P. Howard-Flanders.** 1980. Recognition of duplex DNA containing single-strand regions by RecA protein. Proc. Natl. Acad. Sci. U.S.A. **77**:2569–2573.

366. **Whittier, R. F., and J. W. Chase.** 1981. DNA repair in *E. coli* strains deficient in single-strand DNA binding protein. Mol. Gen. Genet. **183**:341–347.
367. **Whittier, R. F., and J. W. Chase.** 1983. DNA repair properties of *Escherichia coli tif-1, recAo281* and *lexA1* strains deficient in single-strand DNA binding protein. Mol. Gen. Genet. **190**:101–111.
368. **Witkin, E. M.** 1969. Ultraviolet-induced mutation and DNA repair. Annu. Rev. Microbiol. **23**:487–514.
369. **Witkin, E. M.** 1974. Thermal enhancement of ultraviolet mutability in a *tif-1 uvrA* derivative of *Escherichia coli* B/r: evidence that ultraviolet mutagenesis depends upon an inducible function. Proc. Natl. Acad. Sci. U.S.A. **71**:1930–1934.
370. **Witkin, E. M.** 1975. Persistence and decay of thermo-inducible error-prone repair activity in nonfilamentous derivatives of *tif-1 Escherichia coli* B/r: the timing of some critical events in ultraviolet mutagenesis. Mol. Gen. Genet. **142**:87–103.
371. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40**:869–907.
372. **Witkin, E. M.** 1982. From Gainesville to Toulouse: the evolution of a model. Biochimie **64**:549–555.
373. **Witkin, E. M., J. O. McCall, M. R. Volkert, and I. E. Wermundsen.** 1982. Constitutive expression of SOS functions and modulation of mutagenesis resulting from resolution of genetic instability at or near the *recA* locus of *Escherichia coli*. Mol. Gen. Genet. **185**:43–50.
374. **Yamamori, T., and T. Yura.** 1980. Temperature-induced synthesis of specific proteins in *Escherichia coli*: evidence for transcriptional control. J. Bacteriol. **142**:843–851.
375. **Yamamoto, Y., M. Katsuki, M. Sekiguichi, and N. Otsuji.** 1978. *Escherichia coli* gene that controls sensitivity to alkylating agents. J. Bacteriol. **135**:144–152.
376. **Yamamoto, Y., and M. Sekiguichi.** 1979. Pathways for the repair of DNA damaged by alkylating agent in *Escherichia coli*. J. Bacteriol. **171**:251–259.
377. **Yoakum, G. H., A. T. Yeung, W. B. Mattes, and L. Grossman.** 1982. Amplification of the *uvrA* gene product of *Escherichia coli* to 7% of cellular protein by linkage to the P_L promoter pKC30. Proc. Natl. Acad. Sci. U.S.A. **79**:1766–1770.
378. **Youderian, P., S. Bouvier, and M. M. Susskind.** 1982. Sequence determinants of promoter activity. Cell **30**:843–853.
379. **Yukiko, S., T. Kato, and B. W. Glickman.** 1983. *umuC*-mediated misrepair in *Escherichia coli*: extent and specificity of SOS mutagenesis. Mutat. Res. **111**:51–59.